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Supercritical CO_2 extraction of valuable lipophilic compounds from pre-fractionated sour cherry pomace and evaluation of their composition and properties

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ABSTRACT

Valorisation of fruit processing by-products and waste is an important task for increasing the sustainability of agro-food sector. In this study, pitted sour cherry pomace was mechanically pre-fractionated into the 6 different particle size (>10, 4–10, 3–4, 1–3, 0.8–1, <0.8 mm) fractions (9.71–16.41 % proteins, 5.68–12.16 % fat, 62.55–78.39 % carbohydrates) and subjected to supercritical fluid extraction with CO_2 for the recovery of lipophilic constituents. Extract yield depended on fat content and was from 3.38 % to 8.69 %. Linoleic (38.52–47.11 %) and oleic (21.85–39.03 %) were major fatty acids, while triacylglycerols composed of these acids were major in the extracted oils. The concentrations of tocopherols, carotenoids and phytosterols in the extracts were 116.3–432.0, 1218–2564 and 4294–8449 μg/g. Antioxidant activity values were determined for the extracts and solids of initial dry pomace and its residue after extraction. Folin-Ciocalteu Index (basically similar to total phenolic content, TPC), $ABTS^{\bullet+}$-scavenging and oxygen radical absorbance (ORAC) values of extracts were 7.86–8.75 mg of gallic acid equivalents/g, 1.72–6.37 and 35.12–95.49 of mg trolox equivalents/g, respectively. It is the first report on comprehensive characterisation of sour cherry pomace fractions extracted by supercritical CO_2.

1. Introduction

Prunus cerasus L. (common names sour cherry, tart cherry, dwarf cherry) is a native to Europe and Southwest Asia tree, which produces valuable and popular fruits due to their quality attributes and health-promoting compounds. However, nowadays only a small fraction of sour cherry harvests are consumed as fresh fruits due to their more acidic taste compared to sweet cherries; they are processed into various products such as juice, jams, toppings, dried products, wine and other alcoholic drinks. Sour cherry pressing results in the main product, high anthocyanin-rich juice [1] and generates significant amount of pomace, which is mainly discarded as a waste or recycled to lower value products. During the last decades, the interest in valorisation of agro-industrial by-products and waste as a source of various nutrients and bioactive compounds has been steadily increasing. Small fruit (commonly called berries) processing by-products have attracted a special interest due to a high content of valuable nutrients and bioactive compounds, particularly polyphenolic antioxidants and polyunsaturated fatty acids [2,3]. It is important to note tha berry pomace may contain similar or even higher content of bioactive compounds compared with the pulp orthe whole fruit [4].

In general, berry pomaces are highly heterogeneous by-products consisting mainly of skins, residual pulp and seeds; while cherries and other stone fruits contain a big pit inside the fruit. The review on the opportunities of valorisation and application of by-products from *Prunus* genus fruit processing revealed that the reports on sour cherry pomace are rather scarce [5]. The studies on sour cherry pomace composition, functional properties, recovery of polyphenolic antioxidants and valorisation in general have been reviewed in 2018 [6] and 2024 [7]. The skins and residual pulp of many berry species is a good source of polyphenolic antioxidants, while the seeds contain high amount of polyunsaturated fatty acid rich oils and other valuable lipophilic compounds [8,9]. The mass of sour cherry pit in the fruit was reported 14.6 %, while the kernels constituted only 23 % of the pit weight [10]. The content of oil in the kernels, depending on fruit cultivar, was in the range of 17.5–37.1 % [11].

Berry pomace obtained from the fruits with soft seeds may be dried and used for further processing without any mechanical separation,

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while in case of sour cherry pomace, the pits should be separated from the other pomace parts. Dried berry pomace or their mechanically pre-fractionated parts may be milled and used directly in food formulations. For instance, pitted sour cherry pomace was added to muffins as a source of phenolic antioxidants [12]; Gumul et al. added extruded preparations of sour cherry pomace consisting of 13.57 % protein, 3.02 % fat, 10.97 % carbohydrates, and 48.7 % dietary fibre (4.5 % soluble, 44.2 % insoluble) into the gluten-free breads [13]. Pavlović et al. microencapsulated rich in phenolics extracts of pitted sour cherry pomace and evaluated their bio-accessibility during the in vitro gastrointestinal digestion [14]. Toprakçı et al.] extracted sour cherry peels with aqueous 80 % ethanol and encapsulated the extract obtained by ionic gelation in alginate beads [15]. Zhang et al. used sweet and sour cherry pomaces for extracting pectins [16]. Hosseini et al. optimized simultaneous extraction of pectin and phenolics from sour cherry pomace [17]. Sezer et al. focused their study for improving the yield of soluble dietary fibre in sour cherry pomace and characterization of total phenolic content and antioxidant capacity [18].

The recovered from the pomace oils may be used in foods, nutraceuticals, cosmetics and some other products. Sour cherry kernels have been reported as a good source of monounsaturated and polyunsaturated fatty acids, mainly oleic and linoleic [19,20] and bioactive lipophilic constituents such as carotenoids and tocopherols [10]. Phytosterols are also important micronutrients found in sour cherry oils [11, 20,21]. Hence, the lipophilic fraction of sour cherry pomace is rich in various bioactive constituents and therefore development of efficient methods of their recovery is of interest for nutraceutical and cosmeceutical industries.

Various extraction methods have been applied for the recovery of valuable substances from the agro-food materials, mainly attempting to increase the extraction efficiency in terms of the total yield and the concentration of the target compounds. The process highly depends on solvent, particle size, solvent-to-solid ration, temperature and time. The selection of a proper solvent is crucial to increase the solubility and the selectivity of components; it is also important in terms of techno-economic extraction issues and safety. The traditional (conventional) extraction methods, such as maceration, reflux extraction, percolation, and Soxhlet extraction, usually use large volumes of organic solvents and a long extraction time. Therefore, during the last decades many studies have applied more innovative and greener extraction methods of natural compounds, including supercritical fluid extraction with CO_2 (SFE-CO_2), which is particularly attractive for the recovery of lipophilic substances. Supercritical CO_2 has been widely used due to its selectivity for non-polar compounds, low cost, non-toxicity, low critical temperature (31 °C), which is preferable for the heat-sensitive bioactive components [22]. The extracts/oils obtained by this method and the extraction itself might be used in various industries such as food, cosmetics, pharmaceutics and others.

The growing interest in the natural products as well as the need of valorisation of agro-industrial processing by-products and waste fosters further studies on developing efficient methods for the recovery of valuable nutrients from berry pomaces. Moreover, nutraceutical and cosmeceutical industries demand better specified ingredients in terms of their composition and properties. From this point of view, we hypothesize that due to a high heterogenicity of sour cherry pomace, its mechanical pre-fractionation may provide a wider range of extracts with different composition and concentration of bioactive compounds. Therefore, the aim of this study was to recover lipophilic compounds from the pitted and mechanically pre-fractionated sour cherry pomace by the green SFE-CO_2 method and to characterised the products obtained for evaluating the prospects of their application as nutraceutical, functional food and cosmeceutical ingredients. To the best of our knowledge this approach as well as the recovery of nutrients from the pitted sour cherry pomace by using SFE-CO_2 has not been performed previously. It is important to note that for this purpose industrial batch of pomace was used, which may mitigate the upscaling of the applied processes.

2. Materials and methods

2.1. Plant material and chemicals

Industrial sour cherry pomace was donated by the local berry wine producer 'Anykščių vynas' (Anykščiai, Lithuania). The whole pomace immediately after pressing was dried at 60 °C and the unbroken pits were separated from the other parts of the pomace. The pits constituted 74 % of the total mass. The pitted pomace was fractionated by different sieves into 6 fractions (1–6 F), which were ground in a centrifugal high-speed mill Retsch ZM200 (Haan, Germany) using a 0.5 mm sieve and stored in the hermetically closed glass jars in dark, dry and cool room (<18 °C). The overall scheme of experimental work is summarised in Fig. S1, the results of fractionation in Table S1 (Supplementary material).

CO_2 was obtained from Linde Gas (Jonava, Lithuania). *Folin–Ciocalteu* phenolic reagent (FCR), gallic acid (>99 %), 6-hydroxy, 2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox, ≥97 %), 2,2′-azinobis-3-ethylbenzotiazoline-6-sulfonate (ABTS), microcrystalline cellulose, fluorescein (FL) sodium salt, 2,2-azobis(2-amidino-propane) dihydrochloride (AAPH), dimethyl sulfoxide, anhydrous sodium carbonate (98 %), potassium persulfate, potassium dihydrogen phosphate, aqueous sodium hydrogen phosphate were from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Proximate composition

Proximate composition of ground cherry pomace fractions was analysed according to the methods established by the Association of Official Analytical Chemists: for moisture 925.10, crude protein 960.52, crude fat 920.39 and ash 900.02 [23]. The content of carbohydrates was estimated by subtracting the percentage of the determined components from 100 %. All the analyses were performed in triplicate.

2.3. Extraction of pomace

Conventional Soxhlet extraction was used for comparison purpose. Twenty g of pomace were extracted in a Soxhlet apparatus EZ100H (Behr Labor-Technik, Düsseldorf, Germany) with 150 mL of *n*-hexane for 6 h at ~69 °C. The solvent was evaporated in a rotary vacuum evaporator at 40 °C and the residue finally dried at 50 °C.

SFE-CO_2 was carried out in an SFT-110 extractor (Supercritical Fluid Technologies, Newark, DE, USA) using 20 g of ground cherry pomace placed in a 50 mL extraction cell. The static extraction of 10 min was conducted prior to each dynamic extraction. The impact of the dynamic extraction (continuous flow of supercritical CO_2) time (180 min) on the extract yield was determined at the fixed 42.4 MPa pressure and 53 °C temperature, which were previously optimized for cranberry pomace [24]. The temperature was controlled by a surrounding heating jacket, the CO_2 flow rate in the system was controlled manually by the micro-metering valve. The volume of the consumed CO_2 was measured in standard litres per min (SL/min) at standard parameters: $P = 100$ kPa, $T = 20$ °C, $\rho = 0.0018$ g/mL. The extracts were collected in the amber glass bottles at room temperature and kept at −20 °C before further analysis.

2.4. Chromatographic analysis of fatty acids

Fatty acid composition of oil in lipophilic CO_2-extract was analysed by a slightly modified AOAC Ce 2–66 method [23]. Triacylglycerols were saponified and liberated free fatty acids were esterified by heating 0.1 g extract and 0.8 mL of methanolic NaOH (0.5 M) in a round-bottomed flask with condenser until disappearance of the fatty phase. Afterwards, 1 mL of 24 % boron trifluoride/methanol complex

was poured over the top of condenser and boiled for 2 min, then cooled to room temperature. The sample was diluted with 1 mL *n*-hexane, well-shaken and left until the layers separated. The top hexane phase was collected and stored at −20 °C until analysis. For analysis, 100 μL of hexane phase was diluted with 900 μL of GC-grade hexane. Fatty acid methyl esters (FAME) were analysed on a HRGC 5300 (Mega Series, Carlo Erba, Milan, Italy) equipped with flame ionization detector and 100 m length, 0.25 mm inner diameter, 0.20 μm film thickness fused silica capillary column SPTM-2560 (Supelco, Bellefonte, PA, USA). Injector's temperature was 220 °C, detector's 240 °C; oven temperature was programmed from 80 to 240 °C at 4 °C/min and held for 30 min. Helium was used as a carrier gas at a flow rate of 2 mL/min, the amount of injected sample was 1 μL. A mixture of Supelco® 37 FAME mix (Sigma-Aldrich (Steinheim, Germany), was used for identification by comparing retention times. The percentage of each fatty acid was expressed in GC area percent as a mean of 3 replicate runs.

2.5. Determination of triacylglycerols (TAG)

TAGs were analysed as described by Zeb and Murkovic [25] on a Waters AQCUITY ultra performance liquid chromatography system (Waters Corp., Milford, MA, USA) equipped with a hybrid Bruker Daltonics time-of-flight/quadrupole mass detector (UPLC-Q/TOF) (Bremen, Germany). The UPLC equipment consisted of a binary solvent manager, a sample manager, a column heater interfaced with a mass spectrometer equipped with an ESI source operating in a positive mode. Instrument control and data processing were performed by using HyStar software (Bruker Daltonics, Bremen, Germany). Separations were performed on an Acquity BEH, C18 column, 2.1 × 50 mm, particle size 1.7 μm (Waters, Ireland), the autosampler and column oven were maintained at 20 °C and 40 °C, respectively. The analytes were eluted by using an isocratic solvent system consisting of 18 % isopropanol in methanol (0.1 % acetic acid) and 0.05 % of ammonium acetate. The following parameters were selected: flow rate – 0.4 mL/min; separation time – 10 min; fragmentor's potential – 150 V; drying gas temperature – 350 °C; capillary voltage was 4000 V; *m/z* range – 200 –1000.

2.6. Determination of tocopherols and sterols

Saponification was used to convert tocopherol and sterol esters to their free forms prior to HPLC analysis as described elsewhere [26]. The separation of compounds was carried out on a Waters Acquity UPLC consisting of a solvent delivery system, an autosampler, and a column manager (Waters, Milford, MA, USA). An Acquity BEH C18 column (50 × 2.1 mm, 1.7 μm; Waters, Milford, MA, USA) was used for the separation of compounds. Separation was performed using the following gradient: starting with 30 % A (0.1 % formic acid in water) and 70 % B (0.1 % formic acid in acetonitrile), then changing to 100 % B in 1 min and maintaining at 100 % B for 5 min. After that, the gradient was returned to the initial conditions in 0.5 min. The flow rate of the eluents was 0.4 mL/min, the injection volume – 2 μL, and the column temperature – 30 °C. Before each run, the column was equilibrated for 2 min. The mass spectra were recorded on a UPLC-Q/TOF, operating in the APCI positive mode. All the spectral data was collected in the full scan mode in the *m/z* range from 200 to 1200 at a 3 Hz rate. The following parameters of the mass spectrometer were maintained: capillary voltage – 2000 V; corona current – 3000 nA; end plate offset – −500 V; vaporizer temperature – 400 °C; nebulizer gas pressure – 1.6 bar; drying gas flow rate – 8 L/min; drying gas temperature – 200 °C. All the analysed compounds were quantified by their corresponding protonated ion peaks $[M+H]^+$, and single ion chromatograms were extracted with the accuracy of 0.01 *m/z*.

2.7. Analysis of total carotenoids content

The total content of carotenoids was determined spectrophotometrically by using a calibration curve prepared with the β-carotene standard [27]. Briefly, 0.1 g of the extract sample was dissolved in 1 mL of hexane, and the absorbance was measured at 450 nm. The total content of carotenoids was expressed as mg β-carotene equivalents in 100 g of the extract.

2.8. Evaluation of antioxidant potential

The redox reaction with FCR, which is most frequently used for the determination of total phenolic content, was used as a gallic acid equivalence (GAE) method [28]. Considering that strong lipophilic antioxidants measured in this study do not belong to the class of polyphenolic antioxidants, it is indicated as Folin-Ciocalteu Index (FCI). Briefly, 150 μL of sample or methanol (blank) were mixed with 750 μL of diluted in distilled water (1:9, v/v) FCR (2 M), after 3 min of reaction 600 μL of Na_2CO_3 solution (75 g/L) added, left in dark for 2 h and the absorbance measured at 760 nm with Spectronic Genesys 8 spectrophotometer (Thermo Spectronic, Rochester, NY). The results of FCI were expressed as gallic acid equivalents, mg GAE/g of extract (E) and dry pomace weight (DW).

$ABTS^{\bullet+}$-scavenging was measured by decolorisation assay [29]. Firstly, phosphate buffered saline (PBS) solution (75 mmol/L; pH 7.4) was prepared by dissolving 8.18 g NaCl, 0.27 g KH_2PO_4, 1.42 g Na_2HPO_4 and 0.15 g KCl in 1 L of distilled water. The $ABTS^{\bullet+}$ solution was prepared by mixing 50 mL of ABTS (2 mmol/L PBS) with 200 μL $K_2S_2O_8$ (70 mmol/L) and allowing the mixture to stand in the dark at room temperature for 15–16 h before use. The working solution was prepared by diluting the $ABTS^{\bullet+}$ solution with PBS to obtain the absorbance 0.700 at 734 nm. $ABTS^{\bullet+}$ solution (1500 μL) was mixed with 25 μL of sample or methanol (blank), the mixtures left in dark for 2 h and the absorbance measured at 734 nm with Spectronic Genesys 8 spectrophotometer. The $ABTS^{\bullet+}$-scavenging capacity was expressed as trolox equivalents, mg TE/g E and DW.

Oxygen radical absorbance capacity (ORAC) was evaluated by using fluorescein as a fluorescent probe [30]. Twenty-five μL of sample or MeOH (blank) were mixed with 150 μL of fluorescein solution (14 μmol/L) in the 96-well black opaque microplates, pre-incubated for 15 min at 37 °C, followed by a rapid addition of 25 μL of AAPH solution (240 mmol/L). The microplate was immediately placed in the FLUOstar Omega reader (BMG Labtech, Offenburg, Germany), automatically shaken prior to each reading and the fluorescence was recorded every cycle (1 min × 1.1), in total, 120 cycles using 485-P excitation and 520-P emission filters. Raw data were exported from the Mars software to Excel 2003 (Microsoft, Roselle, IL) for further calculations. Antioxidant curves (fluorescence versus time) were first normalized and from the normalized curves, the area under the fluorescence decay curve (AUC) was calculated as $AUC = \sum_{i=1}^{i=120} \frac{f_i}{f_0}$, where f_0 is the initial fluorescence reading at 0 min and f_i is the fluorescence reading at time *i*. The results were expressed as mg TE/g E and DW.

All the above described methods evaluate antioxidant potential of the recovered extracts, while some antioxidants may be strongly bound to the solid matrix and are not extracted. Therefore, in addition to the extracts, antioxidant capacity of solid substances was evaluated by using QUENCHER method proposed by Serpen et al. [31] with some modifications [32]. It evaluates antioxidant capacity directly in the solid material and provides additional important information both on the extraction efficiency and the residual antioxidant capacity of solids after extraction. Briefly, QUENCHER method was applied for the pomace solids using FCR, $ABTS^{\bullet+}$-scavenging and ORAC assays using 10 mg of sample (when necessary, it was diluted with microcrystalline cellulose) or cellulose (blank) as described in detail elsewhere [31,32].

2.9. Statistical data evaluation

Mean values and standard deviations were calculated using MS Excel

2019. All analyses were carried out in triplicate, unless specified otherwise. Significant differences among means were determined by one-way ANOVA, using the statistical package Statgraphics XIX-X64. Tukey's honestly significant difference (HSD) test was used to determine the significant difference among the treatments at $P < 0.05$.

3. Results and discussion

3.1. Proximate composition of cherry pomace fractions

Proximate composition of cherry pomace fractions was found dependent on the particle size (Table 1). The content of crude protein ranged from 9.71 % to 16.41 %, which was at the same level as previously reported (13.57 %) for the unfractionated sour cherry pomace [13]. The highest protein and fat content were determined in the 5 F, while the lowest in the 3 F. Visual examination of the fractions revealed that the minor 5 F (4 % of the total pitted pomace) contained some clearly visible parts of kernels, which were released from the broken pits. It is well-known that the lipids are primarily accumulated in the cherry stone kernels, which remain in the pomace after pressing. Therefore, the amount of lipophilic compounds in the 5 F was almost two times higher than in the other fractions, except for 2 F. The majority of undamaged pits was removed from the pomace; however, some of stones were broken and the highest amount of the released kernels was observed in the 5 F. According to the previously reported data, oil content in the sour cherry kernel was in the range of 17 − 36 % [11,19,33], while pitted cherry pomace contained only 3.02 % of fat [13]. Distribution of proteins among the fractions was more diverse; however, these fractions constituted only 4.0 and 2.2 % in the total mass of the pitted pomace. The highest ash content was determined in 6 F (3.02 %), while the lowest in the 3 F (1.06 %). The moisture content was similar in all fractions (5.76−6.71 %), which is good for SFE-CO_2. Other macro-components, most likely, consisted mainly of carbohydrates and in different fractions ranged from 62.35 % to 78.24 %. It may be mentioned that, as compared with the previously published data, chemical composition may be dependent on fruit variety, climatic growing conditions, soil composition, and the method of analysis [10].

3.2. Supercritical fluid extraction of cherry pomace fractions

Traditionally the lipids may be extracted with hexane, e.g. for the recovery of fat; however, the residual concentration of the solvent in the foodstuff or food ingredient is limited to a max. 1 mg/kg [34]. In addition, hexane is highly flammable and hazardous to the environment substance; therefore, the alternative environment and food-friendly techniques with green solvents such as SFE-CO_2 are preferable for the recovery of ingredients intended for human application. SFE-CO_2 was separately applied for different fractions of cherry pomace and was performed according to Tamkutė et al. at a pressure of 42.4 MPa and a temperature of 53 °C (density of CO_2 at these parameters is 924 kg/m^3), *i. e.*, the parameters which gave the highest yields of lipids [24]. Thus, the highest extract yield (8.69 %) was obtained from the 5 F. In other fractions, except for the 2 F, the extract yield was almost similar and varied from 3.38 % to 3.94 %. The yield of extracts obtained from different fractions of cherry pomace by Soxhlet extraction with hexane in 360 min was by 29–42 % higher (5.04–12.16 %) than by SFE-CO_2 (3.38–8.69 %). However, the recovery of bioactive compounds by SFE-CO_2 in most cases was quantitatively better than achieved with the traditional solvent extraction method.

The effect of extraction time was evaluated by the process kinetics during 180 min for all fractions (Fig. 1). The curves reach plateau after 60 min (1–4 F) and 90 min, (5–6 F), during which, depending on the cherry pomace fraction, approx. 98 % of the total supercritical CO_2 soluble lipophilic fraction yield was recovered. It may be observed that the extraction curves consist of two (5 F and 6 F) and three (1–4 F) zones. In case of 5 F and 6 F the extraction proceeds very fast during 90 min (almost straight kinetics line) and afterwards changes without any observable intermediate phase to the slow period, which is reflected by a smooth curve. These results agree with the process explained by Sovová et al. in SFE-CO_2 of grape seed oil [35]. Meanwhile, for other fractions (1–4 F), three zones may be observed in the extraction kinetics: the first and the fastest zone (0–30 min) is governed by the solubility of lipophilic compounds, the second one (30–60 min) reflects the transition between solubility and mass transfer, while the third zone (60+ min) is governed by a rather slow mass transfer processes from the inner parts of the particles.

To the best of our knowledge, SFE-CO_2 has not been applied for the pitted sour cherry pomace previously. Moreover, only one study used SFE-CO_2 for sour cherry kernels with pure CO_2 at 60 °C and 30 MPa; however, the yields were not indicated in this study [10]. Dimić et al. [36], depending on the process parameters, obtained 2.50 − 13.02 % of cherry (the spiecies not indicated) seed oil, whereas Straccia et al. [37] recovered 5 % oil from the sweet cherry kernels at 45.7 MPa, 46 °C, 120 min, 0.8 mL/min CO_2 flow rate, which was also lower than in a Soxhlet extraction (6.5 %). Woźniak et al. [38] and Adil et al. [39] used a co-solvent ethanol in SFE-CO_2 of phenolic compounds from sour cherry pomace and therefore the results reported in these studies are different as compared to the data obtained in our study.

3.3. Composition of fatty acids and triacylglycerols (TAGs) in the extracts of different cherry pomace fractions

The results of the composition of the fatty acids in different sour cherry pomace extracts recovered by SFE-CO_2 are shown in Table 2. It is evident that unsaturated fatty acids (UFA) dominated in the all fractions: their content was from 70.5 % (6 F) to 83.62 % (5 F) The content of polyUFA (PUFA) was from 41.54 % (6 F) to 60.89 % (5 F), *i.e.* larger than that of monoUFA (MUFA). In general, the differences in fatty acid content between different fractions was not remarkable except for 5 F and 6 F. For instance, the highest difference between MUFA (22.73 %) and PUFA (60.89 %) was in the 5 F, while the highest content of saturated fatty acids (SFA) was determined in the 6 F (18.69 %). In general, the results obtained are in agreement with Kazempour-Samak et al. who reported similar levels of fatty acids in the cold-pressed sour cherry kernel oil [21].

Among individual fatty acids, linoleic (ω-6, 33.54 – 42.06 %) and oleic acids (ω-9, 20.56 % – 34.30 %) were major in the all fractions, while the content of linolenic acid was remarkably lower. However, the

Table 1
Proximate composition of cherry pomace fractions and SFE-CO_2 yields.

Fraction	Proteins, %	Fat%	Minerals, %	Moisture, %	Carbohydrates, %	SFE-CO_2 yields %
1	13.17 ± 0.30^{c}	5.68 ± 0.15^{b}	1.62 ± 0.00^{c}	6.47 ± 0.02^{c}	73.07 ± 0.13^{d}	3.74 ± 0.23^{a}
2	12.54 ± 0.09^{c}	8.31 ± 0.15^{d}	1.20 ± 0.07^{ab}	6.10 ± 0.15^{b}	71.86 ± 0.02^{c}	5.83 ± 0.33^{b}
3	9.71 ± 0.00^{a}	5.04 ± 0.15^{a}	1.06 ± 0.04^{a}	5.80 ± 0.12^{ab}	78.39 ± 0.08^{f}	3.38 ± 0.10^{a}
4	11.38 ± 0.04^{b}	5.89 ± 0.14^{b}	1.37 ± 0.00^{b}	5.76 ± 0.05^{a}	75.61 ± 0.14^{e}	3.90 ± 0.04^{a}
5	16.41 ± 0.32^{e}	12.16 ± 0.11^{e}	2.33 ± 0.00^{d}	6.55 ± 0.04^{c}	62.55 ± 0.17^{a}	8.69 ± 0.29^{c}
6	15.27 ± 0.15^{d}	6.75 ± 0.14^{c}	3.02 ± 0.10^{e}	6.71 ± 0.02^{c}	68.26 ± 0.22^{b}	3.94 ± 0.32^{a}

The shown results are means ± SD of three independent experiments. [a,b] Different letters within the same column indicate statistical differences (one-way ANOVA, $P < 0.05$).

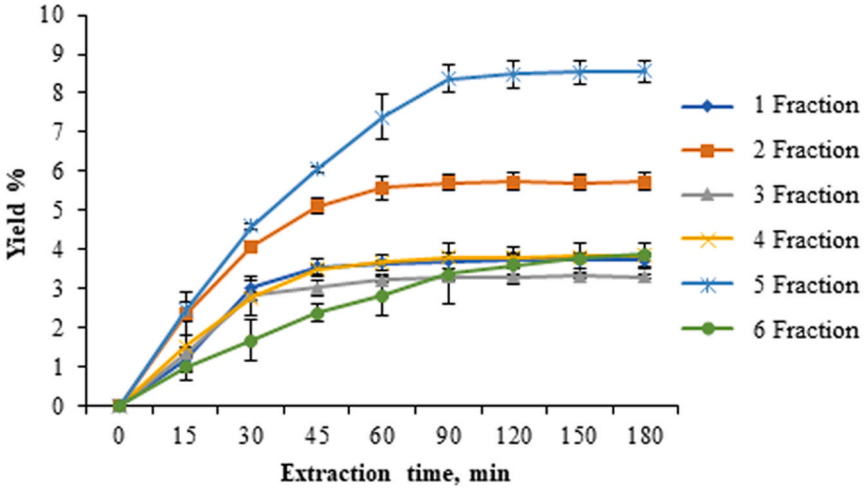

Fig. 1. Kinetics of SFE-CO_2 process (42.4 MPa and 53 °C) of different cherry pomace fractions: the measured values are the means ± SD of three independent experiments.

Table 2
The composition of fatty acids and triacylglycerols (TAGs) of cherry pomace oils in SFE-CO_2 extracts from different fractions (F).

Compound	1 F	2 F	3 F	4 F	5 F	6 F
	Fatty acids, %					
P: C16:0	$_{D}9.34\pm0.00^{a}$	$_{C}9.09\pm0.13^{a}$	$_{D}9.40\pm0.42^{a}$	$_{D}10.43\pm0.13^{a}$	$_{C}8.84\pm0.60^{a}$	$_{D}14.86\pm0.76^{b}$
PO: C16:1	$_{A}0.52\pm0.01^{b}$	$_{A}0.53\pm0.01^{ab}$	$_{A}0.53\pm0.02^{ab}$	$_{A}0.57\pm0.00^{b}$	$_{A}0.28\pm0.01^{a}$	nd
S: C18:0	$_{B}1.88\pm0.01^{a}$	$_{B}2.04\pm0.05^{bc}$	$_{C}2.05\pm0.02^{cd}$	$_{B}2.12\pm0.00^{d}$	$_{B}1.85\pm0.03^{ab}$	$_{B}3.12\pm0.03^{e}$
O: C18:1n9c	$_{E}34.33\pm0.33^{c}$	$_{D}39.03\pm0.25^{f}$	$_{E}37.10\pm0.10^{e}$	$_{E}35.69\pm0.08^{d}$	$_{E}21.85\pm0.02^{a}$	$_{E}32.05\pm0.06^{b}$
L: C18:2n6c	$_{F}44.68\pm0.34^{b}$	$_{E}47.11\pm0.52^{cd}$	$_{F}45.71\pm0.15^{cd}$	$_{F}44.10\pm0.32^{bc}$	$_{F}44.72\pm0.26^{d}$	$_{F}38.52\pm0.13^{a}$
A: C20:0	$_{A}0.68\pm0.01^{b}$	$_{A}0.84\pm0.08^{bc}$	$_{A}0.85\pm0.03^{c}$	$_{A}0.76\pm0.02^{bc}$	$_{A}0.40\pm0.03^{a}$	$_{A}1.06\pm0.06^{d}$
Ln: C18:3n6	$_{A}1.05\pm0.01^{b}$	nd	$_{A}0.56\pm0.00^{a}$	nd	$_{D}9.84\pm0.11^{c}$	$_{A}0.63\pm0.00^{a}$
E: C20:1	nd	nd	$_{A}0.41\pm0.02^{a}$	$_{A}0.63\pm0.00^{b}$	$_{A}0.58\pm0.05^{b}$	nd
Ln: C18:3n3	$_{C}2.91\pm0.01^{c}$	$_{AB}1.32\pm0.05^{a}$	$_{BC}1.92\pm0.01^{b}$	$_{C}3.34\pm0.03^{d}$	$_{D}10.18\pm0.01^{f}$	$_{C}8.55\pm0.08^{e}$
N: C24:1	nd	nd	$_{B}1.46\pm0.07^{a}$	$_{B}2.25\pm0.04^{b}$	$_{B}1.45\pm0.15^{a}$	$_{A}1.21\pm0.11^{a}$
	TAGs, %					
LLLn	18.08 ± 0.29^{b}	17.04 ± 0.07^{a}	17.77 ± 0.09^{ab}	17.87 ± 0.10^{b}	20.08 ± 0.36^{c}	19.50 ± 0.11^{c}
LnLnLn	0.56 ± 0.01^{b}	0.23 ± 0.00^{a}	0.27 ± 0.00^{a}	0.31 ± 0.00^{a}	3.43 ± 0.09^{c}	0.95 ± 0.02^{b}
SLO	9.32 ± 0.03^{c}	9.85 ± 0.06^{d}	9.40 ± 0.04^{cd}	9.60 ± 0.09^{cd}	3.72 ± 0.05^{a}	7.52 ± 0.24^{b}
NK1	4.91 ± 0.02^{a}	4.62 ± 0.14^{a}	4.71 ± 0.02^{a}	4.68 ± 0.04^{a}	5.79 ± 0.03^{b}	6.05 ± 0.15^{b}
PLS	3.22 ± 0.00^{b}	3.29 ± 0.07^{b}	3.34 ± 0.07^{b}	3.44 ± 0.04^{b}	1.42 ± 0.07^{a}	3.31 ± 0.14^{b}
LLnLn	3.59 ± 0.02^{ab}	3.12 ± 0.15^{a}	3.84 ± 0.08^{bc}	4.27 ± 0.06^{c}	10.92 ± 0.06^{e}	6.89 ± 0.05^{d}
OLL	18.90 ± 0.57^{bc}	20.14 ± 0.26^{c}	19.43 ± 0.25^{bc}	18.67 ± 0.28^{b}	15.18 ± 0.22^{a}	16.165 ± 0.29^{a}
PLnLn	0.53 ± 0.01^{d}	0.24 ± 0.01^{a}	0.42 ± 0.02^{b}	0.47 ± 0.01^{c}	1.95 ± 0.01^{f}	0.65 ± 0.00^{e}
PLL	5.04 ± 0.17^{b}	4.55 ± 0.03^{a}	4.81 ± 0.04^{ab}	4.75 ± 0.09^{ab}	6.05 ± 0.01^{c}	6.02 ± 0.02^{c}
OLnL	11.59 ± 0.16^{a}	12.06 ± 0.29^{a}	11.49 ± 0.40^{a}	11.82 ± 0.20^{a}	19.00 ± 0.04^{b}	12.01 ± 0.13^{a}
PLO	7.09 ± 0.07^{b}	7.10 ± 0.16^{b}	7.06 ± 0.03^{b}	6.95 ± 0.08^{b}	3.83 ± 0.09^{a}	6.85 ± 0.17^{b}
SLL	17.17 ± 0.45^{c}	17.75 ± 0.22^{c}	17.44 ± 0.02^{c}	17.16 ± 0.17^{c}	8.64 ± 0.03^{a}	14.43 ± 0.08^{b}

*nd – not detected; Palmitic acid (C16:0), Palmitoleic acid (C16:1), Stearic acid (C18:0), Oleic acid (C18:1n9c), Linoleic acid (C18:2n6c), Arachidic acid (C20:0), γ-Linolenic acid (C18:3n6), cis-11-Eicosenoic acid (C20:1), Linolenic acid (C18:3n3), Nervonic acid (C24:1). The results are expressed as mean ± standard deviation of triplicate determinations. a,b Different letters within the same row indicate statistical differences (one-way ANOVA, $P < 0.05$); A,B Different letters within the same row indicate statistical differences (one-way ANOVA, $P < 0.05$). L – linoleic acid; Ln – linolenic acid; O – oleic acid; P – palmitic acid; S – stearic acid; NK1 – not identified TAG. Peak area in arbitrary units x 105. a,b Different letters within the same row indicate statistical differences (one-way ANOVA, $P < 0.05$).

5 F may be recognised as an exceptional fraction: it contained 9.84 % of γ- (ω-6) and 10.18 % of α-linolenic (ω-3) acids. The dominant SFAs in the all fractions of cherry pomace were palmitic (7.99 – 12.94 %) and stearic (1.66 – 2.72 %). These results are in agreement with Popa et al. who reported that sour cherries kernel oil contained 11 % of palmitic acid and remarkably lower amount of arachidic (0.9 %) and myristic (0.5 %) acids, but a higher content of stearic acid (6.4 %) [40]. Yilmaz & Gökmen reported the composition of fatty acids of sour cherry kernels oil obtained by SFE-CO_2 [10]. Their results showed that the dominant fatty acids were the same as in our study, namely palmitic (7.24 %), stearic (1.33 %), oleic (44.99 %), linoleic (41.81 %) and linolenic (4.63 %). Some differences in fatty acid composition could be due to the different composition of sour cherry fractions; in the above-mentioned studies, only the kernels were used, while in our study the pomace contained only small amount of kernel and shell fragments. It may be noted that Górnaś et al. [11], Kazempour-Samak et al. [21] and Atik et al. [20] reported the presence of α-eleostearic acid, while it was not found by Yilmaz & Gökmen [10] and our study.

The sum of linoleic and linolenic acids was found to exceed 42 % in the all extracts isolated from the sour cherry pomace fractions. These fatty acids are classified as ω-6 and ω-3 PUFA and are very important for the human diet. Moreover, the nutritionists recommend to increase the consumption of ω-3 and ω-6 fatty acids, while keeping their recommended ratio of 1–3.

The composition of TAGs is important for physical, technological and nutritional properties of fats. For instance, fat plasticity and melting point depends both on fatty acid composition and their position in the TAG. In our study, twelve triacylglycerols were characterised semi-

quantitatively by their percentage composition in the SFE-CO_2 extracts of different cherry pomace fractions using UPLC-Q/TOF (Table 2). Three types of TAGs were identified consisting of one (LnLnLn), two (LLLn, LLnLn, OLL, PLnLn, PLL, SLL) or three different fatty acids (SLO, PLS, PLO). The major TAGs in the SFE-CO_2 extracts were LLLn, OLL, OLnL, SLL, SLO, PLO, while LnLnLn, NK1 (not identified), PLS, LLnLn, PLnLn and PLL were the minor ones. In general, the differences in TAGs composition between the fractions were not remarkable, except for the 5 F, which contained significantly higher percentage of highly unsaturated TAGs composed of 2 and even 3 linolenic acids. For instance, the content of trilinolenate (LnLnLn) in this fraction was 3.43 %, while in the others it was from 0.23 − 0.95 %. Similarly, the content of LLnLn in this fraction was up to 3.5 times higher than in the other fractions. It may be observed that the composition of TAGs agrees with the percentage composition of fatty acids (Table 2).

The information on TAG composition of sour cherry oils is rather scarce and, to the best of our knowledge, there is no data on the identification of TAGs in the oil of the sour cherry pomace oil extracted by SFE-CO_2. The predominant TAGs in the sour cherry pit kernel oil, which was extracted with hexane and contained 47.62 % of oleic acid, were triolein (OOO – 16.83 %) followed by OLO – 16.64 %, LLO – 13.20 %, OLP – 7.25 %, OOP – 6.49 % and LElL – 6.16 % [19]; whereas OLL (20.44 %), OOL (16.99 %), (LLL) (8.20 %), LLEl (7.28 %), PLO (7.24 %) were the most abundant TAGs in the oil of the wild sour cherry (*Cerasus vulgaris* Miller) kernel [21]. It may be noted that α-eleostearic acid (El) was found in reasonable amounts in the previously studied sour cherry kernels, while in our study the content of kernels in pomace was very low. In general, the differences in TAGs composition probably might be explained by different cultivars, anatomical parts, growing conditions, and different extraction methods, which have been applied for their recovery.

The composition of TAGs in oil is highly dependent on the composition and ratio of pomace with the seeds. The TAGs of berry pomace are usually composed of higher-saturation fatty acids, while the seed oil is more unsaturated. According to the results of our study, a higher amount of unsaturated TAGs and less of saturated TAGs were determined in the 5 F fraction compared to other fractions obtained with SFE-CO_2. In case of a Soxhlet extraction, these differences were negligible. The obtained results might be explained by the fact that the cherry seed oil obtained by SFE-CO_2 had a higher content of PUFAs.

3.4. Lipophilic antioxidants – tocopherols and carotenoids in different cherry pomace fractions

The unsaponifiable matter in oils contains components that play a critical role for their oxidative stability and shelf-life. To these components belong triterpenes and aliphatic alcohols, tocopherols, pigments, β-carotene, squalene and sterols.

Tocopherols, which are found in various natural sources, act as antioxidants protecting unsaturated fatty acids from oxidative damage. These components are synthesized only in photosynthetic organisms and in human nutrition act as vitamin E. Tocopherols are well known for their antioxidant and anti-inflammatory properties; α-tocopherol (vitamin E) being the strongest and most widely studied antioxidant among tocols, particularly in terms of its effects on the immune system, anti-inflammatory, and antitumor activities. However, the review of health benefits of other natural forms of vitamin E (γ- and δ-tocols) revealed their superior cancer-preventive activities compared to α-tocopherol; for instance, inclusion of γ-tocopherol into the diet may prevent cancer (e.g., prostate) and cardiovascular disease [41]. It is also important to note that their bioavailability and metabolism are different.

Different fractions of sour cherry pomace oils obtained by SFE-CO_2 showed a great variation in the total content and composition of tocopherols. In this study, the total tocopherol content was lower in the hexane extracts, 81.84 (2 F) – 205.84 μg/g (4 F), than in the extracts recovered by SFE-CO_2, 141.85 (6 F) – 432.0 μg/g (5 F) in the all fractions of cherry pomace. As reported by Yilmaz & Gökmen [16], the total tocopherol concentration in the hexane extract (428.62 mg/L) was higher than in the SFE-CO_2 extract (312.15 mg/L) of cherry kernels, while Matthäus & Özcan [33] reported 240.2 mg/kg in the kernel oil of *P. cerasus* extracted by petroleum ether. The results obtained in our study using pitted sour cherry pomace fractions are of the same order; however, most probably, higher amounts of tocopherols are accumulated in the seed (kernels). For instance, Kazempour-Samak et al. reported 832.5 mg of tocopherols in one kg of cold pressed sour cherry oil [21].

Four tocopherols (α, β, γ and δ) were identified and quantified in the sour cherry oils obtained by different extraction methods. Their concentrations in the SFE-CO_2 extracts of different sour cherry pomace fractions were as follows (in μg/g oil): α-tocopherol, 81.39 (2 F) – 195.89 (4 F), β+γ-tocopherols, 45.60 (1 F) – 272.00 (5 F), and δ-tocopherol, 11.38 (2 F) – 27.94 (5 F) (Table 3). Among the identified and quantified tocopherols vitamin E (α-tocopherol) was the most abundant with an average concentration of 134.3 μg/g, contributing to 51.76 % of the total concentration in the SFE-CO_2 extracts, followed by the sum of chromatographically not separated β+γ-tocopherols and δ-tocopherol. Cherry pomace oils from the 5 F, although being low in α-tocopherol content compared to the oils obtained from other fractions by SFE-CO_2, showed the highest total tocopherols concentration, followed by the oil from the 4 F. The oil from the 5 F exhibited the highest concentration of γ-tocopherol (272 μg/g of oil). The SFE-CO_2 oils contain certain amounts of δ-tocopherol with some variations between different fractions, 11.38 (2 F) – 27.94 μg/g (5 F).

Kazempour-Samak et al. [21] reported in the wild sour cherry kernel 325, 470 and 37.5 mg/kg oil of α-, γ-, and δ -tocopherol, respectively; while Górnaś et al. [11] quantified all four tocopherols in different sour cherry cultivars, γ-tocopherol also being the most abundant with the values in the range of 89.1 − 133.3 mg/100 g oil. Korlesky et al. reported remarkably lower concentration of the major γ-tocopherol in cherry pit oil, only 40 mg/100 g [19]. γ-Tocopherol as the major representative was determined in most *Prunus* species kernel oils [8,20, 42,43], except for *Prunus persica* kernel oil, where α-tocopherol was dominant with 37.3 mg/100 g [26], and 175.4 – 187.5 mg/100 g oil [44]. Tocopherols were not determined in the other sour cherry pomace fractions previously. Our study has revealed that, in contrary to kernel oil, α-tocopherol is a dominating constituent, except for the 5 F, which, as mentioned above, contained visible amount of kernels. It should be mentioned that the concentration of tocopherols in the recovered extracts may highly depend on the extraction pressure; for instance, it was reported that at 20 MPa and 60 °C the concentration of tocopherols in the recovered Moroccan argan oil [45] due to the lower overall yield was approx. 3-fold higher than in the oils recovered at 30 and 40 MPa (high overall yields). In addition, proper selection of a CO_2/biomass mass ratio may also increase the concentration of tocopherols [46]. Consequently, it may be reasonably hypothesized that tocopherol-rich fractions may also be obtained from cherry pomace by further optimization of process parameters. However, this task was beyond the objectives of our study.

Carotenoids are naturally occurring pigments found in plants with different bioactivities, e.g. β-carotene is a well-established provitamin A [47]. The intake of foods containing these micronutrients could be beneficial against certain types of cancer, may help to prevent heart or other diseases [48]. In addition, carotenoids are strong antioxidants acting by different mechanisms. As reported in Table 3, the total carotenoid content in the SFE-CO_2 extracts ranged from 1.22 (5 F) to 2.56 mg β-carotene/g (6 F). For comparison, Tamkutė et al. reported 11.98 mg β-carotene in 100 g of SFE-CO_2 extract recovered from cranberry pomace [24]. The solubility of carotenoids in supercritical CO_2 is highly dependent on its density, properties of the solute and the solubility of the main components of the system [49].

Table 3
The content of bioactive lipophilic microconstituents in different cherry pomace extracts obtained by SFE-CO_2.

Compounds		1 F	2 F	3 F	4 F	5 F	6 F
Tocopherols							
β+γ-tocopherols, μg/g	Extract	45.60±1.11^{a}	49.40±0.86^{a}	83.51±1.10^{b}	135.8±7.5^{c}	272.0±0.7^{d}	48.85±0.26^{a}
	DW	1.71±0.04^{a}	2.88±0.05^{b}	2.82±0.04^{b}	5.30±0.29^{c}	23.64±0.06^{d}	1.92±0.01^{a}
δ-tocopherol, μg/g	Extract	15.37±1.05^{ab}	11.38±1.06^{a}	17.90±0.56^{b}	16.19±0.64^{ab}	27.94±1.21^{c}	27.08±2.42^{c}
	DW	0.57±0.04^{a}	0.66±0.06^{a}	0.61±0.02^{a}	0.63±0.02^{a}	2.43±0.11^{c}	1.07±0.10^{b}
α-tocopherol, μg/g	Extract	86.59±2.93^{bc}	55.35±0.30^{a}	80.49±5.48^{b}	177.9±0.14^{e}	132.0±2.37^{d}	93.22±1.42^{c}
	DW	3.24±0.11^{b}	3.23±0.02^{b}	2.72±0.19^{a}	6.94±0.01^{d}	11.47±0.21^{e}	3.67±0.06
Total, μg/g	Extract	147.6±2.9^{b}	116.3±0.4^{a}	181.9±4.9^{c}	329.9±8.3^{d}	432.0±0.4^{e}	169.8±1.5^{c}
	DW	5.52±0.11^{a}	6.78±0.02^{b}	6.15±0.17^{b}	12.87±0.32^{c}	37.54±0.04^{d}	6.69±0.06^{b}
Carotenoids							
μg/g	Extract	2528±13^{e}	1695±5^{b}	2051±3^{c}	2102±8^{d}	1218±3^{a}	2564±6 ^{f}
	DW	94.54±0.48^{c}	98.79±0.30^{d}	69.33±0.10^{a}	81.96±0.30^{b}	105.8±0.2 ^{f}	101.0±0.3^{e}
Phytosterols							
Campesterol, μg/g	Extract	728.7±15.7^{c}	358.3±2.8^{a}	347.8±21.6^{a}	468.2±2.2^{b}	496.0±24.7^{b}	368.7±8.5^{a}
	DW	27.25±0.59^{d}	20.89±0.17^{c}	11.76±0.73^{a}	18.26±0.08^{bc}	43.11±2.15^{e}	14.53±0.33^{ab}
Cholestan−3-ol, μg/g	Extract	41.46±1.23^{c}	38.68±1.13^{c}	32.49±0.86^{b}	24.31±0.92^{a}	34.42±0.54^{b}	34.06±1.21^{b}
	DW	1.55±0.05^{c}	2.26±0.07^{d}	1.10±0.03^{a}	0.95±0.04^{a}	2.99±0.05^{e}	1.34±0.05^{b}
Stigmasterol, μg/g	Extract	215.7±9.0^{b}	141.4±12.1^{a}	113.7±16.5^{a}	146.1±2.8^{a}	198.5±3.7^{b}	148.9±10.7^{a}
	DW	8.07±0.34^{c}	8.24±0.70^{c}	3.84±0.56^{a}	5.70±0.11^{b}	17.25±0.32^{d}	5.87±.0.42^{b}
Ergosterol, μg/g	Extract	0.00±0.00^{a}	0.00±0.00^{a}	19.08±0.45^{c}	15.41±1.01^{b}	0.00±0.00^{a}	0.00±0.00^{a}
	DW	0.00±0.00^{a}	0.00±0.00^{a}	0.74±0.02^{c}	0.52±0.03^{b}	0.00±0.00^{a}	0.00±0.00^{a}
β-sitosterol, μg/g	Extract	7271±375^{c}	3756±331^{a}	5133±543^{b}	4543±354^{ab}	3620±247^{a}	3990±130^{ab}
	DW	271.9±14.0 ^{cd}	219.0±19.3^{bc}	173.5±18.3^{ab}	177.2±13.8^{ab}	314.6±21.4^{d}	157.2±5.1^{a}
Total	Extract	8449±218^{c}	4294±320^{a}	5646±549^{b}	5197±361^{ab}	4349±276^{ab}	4542±131^{ab}
	DW	316.0±8.1^{c}	250.3±18.7^{b}	190.9±18.5^{ab}	202.6±14.1^{ab}	377.9±23.9^{c}	178.9±5.1^{a}

The shown results are means ± SD of two independent experiments; ^{a,b} Different letters within the same row indicate statistical differences (one-way ANOVA, $P < 0.05$)

3.5. Phytosterols in different cherry pomace fractions

The main biologically relevant phytosterols found in plants are sitosterol, campesterol, stigmasterol and brassicasterol. The consumption of phytosterols is associated with various health benefits, including the potential to reduce total and low-density lipoprotein cholesterol levels and thereby decreasing the risk of cardiovascular diseases, anti-obesity, anti-diabetic, anti-microbial, anti-inflammatory, and immunomodulatory and anticancer effects [50]. Five sterols, namely campesterol, cholestan-3-ol, stigmasterol, ergosterol, β-sitosterol were identified and quantified in different fractions of cherry pomace extracts obtained by SFE-CO_2. β-Sitosterol was the major compound in the all extracts, constituting 87 – 91 % of the total quantified phytosterols, which agrees with the data of Kazempour-Samak et al. [21] and Atik et al. [20] who determined that β-sitosterol constituted 83.55 % and 88.69 % (6018 ppm), respectively, in the fraction of phytosterols from sour cherry kernels. It is not surprising, since β-sitosterol, along with stigmasterol and campesterol, is the most abundant phytosterol in the plant origin lipids [51]. According to Górnaś et al. [11] β-sitosterol concentration in the oils of the sour cherry kernels of different cultivars varied from 241.0 to 852.8 mg/100 g oil, while in the pit oil of the sour cherry (*P. cerasus*) it was 3610 ppm [19]. In our study, the prefractionation of cherry pomace had significant effect; higher concentration of these components was observed when SFE-CO_2 was applied, except for the 4 F and 5 F.

Other quantitatively major sterols in the SFE-CO_2 extracts were campesterol and stigmasterol. Campesterol varied from 347.8 (3 F) to 728.7 μg/g (1 F), while stigmasterol concentration was remarkably lower, from 113.7 (3 F) to 215.7 μg/g (1 F). The content of campesterol and stigmasterol in nine different varieties of apricots was reported in the range of 11.17 – 15.50 and 1.39 – 3.81 mg/kg oil [52], in the pits of sour cherries it was 159 and 7.2 ppm [19], respectively, while in different varieties of sour cherry kernel oil it was 7.6 – 41.6 mg/100 g oil and 0 – 12.2 mg/100 g oil, respectively [11].

The other detected sterols (cholestan-3-ol and ergosterol) were minor constituents: ergosterol was detected only in two fractions, 4 F and 5 F. The oils of SFE-CO_2 extracts contained quantified amounts of cholestan-3-ol with only a small variation between different fractions, 17.61 (2 F) – 33.21 μg/g (4 F) and 24.31 (4 F) – 41.46 μg/g (1 F), respectively. To the best of our knowledge, cholestan-3-ol and ergosterol are reported in the cherry pomace extracts for the first time.

3.6. Antioxidant potential of extracts and solids

It is believed that dietary antioxidants may mitigate oxidative damage of important biomolecules by neutralising excessive reactive oxygen and nitrogen species and thereby reducing the risk of the development of various inflammation related diseases, including cardiovascular disorders and cancer [53]. Natural antioxidant and antimicrobial agents from the cheap residual sources are also considered as attractive constituents, which may serve as the alternatives of oxidation and microbial spoilage inhibiting synthetic additives, which are used in various foods for increasing their shelf-life and safety [54–57]. Polyphenolics, which are usually recovered using medium and higher polarity solvents, are the major group of fruit antioxidants; however, lipophilic fractions may also contain radical scavengers (mainly tocopherols and carotenoids), which exert their antioxidant activity by different mechanisms.

In this study, the antioxidant potential of SFE-CO_2 extracts obtained from different fractions of sour cherry pomace as well as the solids, including initial material and the residues remaining after extraction, was evaluated by the free different assays, namely redox reaction with FCR, $ABTS^{\bullet+}$-scavenging, and ORAC. The two former assays are based on a single electron transfer, while the latter one on the peroxyl radical absorbance, which is better related to the processes happening in biosystems. According to the experts in this area all selected assays provide supplementary data on antioxidant potential of plant-based extracts and foods in general [58].

It may be observed that the differences between the extract FCI were not remarkable; the measured values were in the range of 7.86 – 8.64 mg GAE/g, while the differences in the recovery of the compounds participating in the redox reaction with FCR from the fraction gram of dry weight were more remarkable, 0.27 – 0.71 mg GAE/g DW. The values of $ABTS^{\bullet+}$-scavenging capacity and ORAC for the extracts were in a wider range, 1.72 (5 F) – 6.37 (3 F) and 35.12 (5 F) – 95.49 (6 F) mg TE/g, respectively (Table 4). It may be observed that the extracts of 5 F and 6 F contained the lowest and the highest concentration of carotenoids; however, clear correlations, which are usually established between

Table 4
Total phenol content and antioxidant activity of cherry pomace and residues after extractions and different cherry pomace extracts obtained by SFE-CO_2.

Fraction	FCI, mg GAE/g of product	FCI, mg GAE/g DW	$ABTS^{\bullet+}$, mg TE/g of product	$ABTS^{\bullet+}$, mg TE/g DW	ORAC, mg TE/g of product	ORAC, mg TE/g DW
Sour cherry pomace						
1	14.16±1.56^{b}	15.14±1.66^{b}	44.31±2.68^{ab}	47.38±2.86^{ab}	17.99±1.16^{ab}	19.24±1.24^{ab}
2	8.76±0.71^{a}	9.32±0.75^{a}	39.43±2.99^{a}	42.00±3.18^{a}	16.42±1.26^{a}	17.49±1.35^{a}
3	11.61±0.53^{b}	12.33±0.56^{b}	35.80±2.18^{a}	38.00±2.32^{a}	22.61±1.34^{c}	24.00±1.42^{c}
4	11.36±0.35^{ab}	12.06±0.38^{ab}	53.42±5.78^{b}	56.69±6.14^{b}	16.85±1.09^{ab}	17.88±1.16^{ab}
5	12.64±1.19^{b}	13.52±1.27^{b}	51.09±3.18^{b}	54.68±3.40^{b}	49.48±1.64^{d}	52.95±1.40^{d}
6	25.59±1.53^{c}	27.43±1.64^{c}	106.2±5.5^{c}	113.9±5.9^{c}	19.29±1.32^{b}	20.68±1.42^{b}
Residues after SFE-CO_2						
1	15.83±0.80^{c}	14.25±0.72^{d}	52.42±2.36^{b}	47.19±2.13^{ab}	24.52±2.82^{b}	21.08±2.54^{b}
2	7.35±0.78^{a}	6.50±0.69^{a}	33.84±1.70^{a}	29.92±1.50^{a}	14.26±1.23^{b}	12.61±1.09^{a}
3	8.85±0.70^{a}	8.05±0.64^{b}	33.31±0.46^{a}	30.32±0.42^{a}	17.82±0.45^{b}	16.22±0.41^{a}
4	11.70±0.59^{b}	10.59±0.54^{c}	45.26±1.91^{ab}	40.99±1.73^{b}	13.57±0.36^{a}	12.29±0.33^{a}
5	13.31±0.54^{b}	11.36±0.46^{c}	54.15±6.15^{b}	63.27±5.25^{b}	45.78±2.41^{c}	40.00±2.06^{c}
6	22.52±0.80^{d}	20.18±0.72^{e}	101.5±7.6^{c}	90.97±6.78^{c}	27.12±2.69^{b}	23.26±2.42^{b}
SFE-CO_2 extract						
1	8.75±0.22^{c}	0.33±0.01^{bc}	4.26±0.10^{c}	0.16±0.00^{b}	54.92±2.73^{b}	2.06±0.10^{ab}
2	8.19±0.33^{abc}	0.48±0.02^{d}	4.35±0.34^{c}	0.25±0.02^{e}	36.85±1.97^{a}	2.15±0.11^{ab}
3	7.94±0.63^{ab}	0.27±0.02^{a}	6.37±0.22^{e}	0.22±0.01^{d}	54.53±1.42^{b}	1.84±0.05^{a}
4	7.86±0.47^{a}	0.31±0.02^{b}	4.85±0.13^{d}	0.19±0.01^{c}	58.99±3.11^{b}	2.30±0.12^{b}
5	8.17±0.12^{abc}	0.71±0.01^{e}	1.72±0.15^{a}	0.15±0.01^{b}	35.12±1.60^{a}	3.05±0.14^{c}
6	8.64±0.33^{bc}	0.34±0.01^{c}	2.61±0.17^{b}	0.10±0.01^{a}	95.49±4.92^{c}	3.76±0.19^{d}

The shown results are means ± SD of three independent experiments; a,b Different letters within the same column for the same material indicate statistical differences (one-way ANOVA, $P < 0.05$).

the content of polyphenolic antioxidants and antioxidant capacity values for the higher polarity extracts of berries [59], were not so evident for lipophilic extracts produced in our study. Remarkably higher antioxidant activity values were determined by using QUENCHER method for the unextracted pomace fractions. Moreover, these values in most cases only slightly reduced in the residues after SFE-CO_2, except for the 1 F, when all antioxidant potential values were higher in the residue than in the initial material. It may be explained by the better availability of functional groups present in this fraction, which are reacting with FCR or $ABTS^{\bullet+}$, after high pressure treatment and defatting *via* SFE-CO_2. These findings show that insoluble in supercritical CO_2 polyphenolic compounds, which are usually evaluated by these *in vitro* methods, are the main antioxidants in sour cherry pomace and remain in it after SFE-CO_2.

Comparing with the previously reported data on antioxidant capacity of lipophilic sour cherry kernel extracts, Stryjecka et al. using the TEAC assay ($ABTS^{\bullet+}$-discoloration) determined in the oils extracted with petroleum ether from eight sour cherry cultivars remarkably lower values, 0.382 – 0.432 mg TE/g oil [42]. These values are similar to the results reported by Uluata & Özdemir [43]: the antioxidant capacity in $ABTS^{\bullet+}$-discoloration assay of extracted with hexane oil was 0.387 mg TE/g (1.72 – 6.37 mg TE/g in our study). Wu et al. reported only 0.16 – 0.17 mg TE/g for the extracts of peach kernels isolated using different nonpolar organic solvents [60]. These differences indicate that pitted sour cherry fractions are potentially remarkably stronger antioxidants. Atik et al. using $DPPH^{\bullet}$-scavenging assay determined 465 mg TE in g of *P. cerasus* kernel extract; however, in this case they used for extraction high polarity methanol, which efficiently solubilises strong polyphenolic antioxidants [20]. The FCI values ("total phenol content") determined in our study were also remarkably higher compared to other studies. For instance, according to Stryjecka et al. the total phenol content in the oils from eight different sour cherry cultivars varied from 19.6 to 28.5 μg GAE/g [42]. To the best of our knowledge ORAC was not previously reported for any lipophilic extracts.

4. Conclusion

Mechanical separation of pitted sour cherry pomace into different particle size fractions enabled to obtain the products with significantly different proximate composition, particularly regarding the content of important nutrients – proteins and lipids. Supercritical fluid extraction of the separated fractions with carbon dioxide yielded 3.38 – 8.69 % of lipophilic extracts with different composition of fatty acids, triacylglycerols, tocopherols, carotenoids, and phytosterols, which, in general, supports our hypothesis. The differences in radical scavenging capacity of the extracts obtained were also significant and, in general, remarkably higher compared with the previously reported data for oils extracted from sour cherry kernels by using conventional methods. Consequently, inexpensive mechanical separation of industrial sour cherry pomace before its further application in foods or recovery of natural high value lipophilic ingredients with specified composition might be of interest for nutraceutical and cosmeceutical industries. In addition, the use of food and environment friendly extraction method would provide some definite extra value for the products obtained. It may be noted that health claims have been approved for oleic, linoleic and α-linolenic acids, α-tocopherol (vitamin E) and plant sterols [61]. However, further studies on bioactivities and health effects, such as skin protection, inflammation and other biomarkers, should be performed for identifying specific applications of the products obtained. In addition, for up-scaling and commercialization of the ingredients Life Cycle and Techno-Economic Assessment (LCA-TEA) would be important issues. Finally, this study expands our scientific knowledge on lipophilic constituents of pitted sour cherry pomace, which until now was rather scarce.

CRediT authorship contribution statement

Vita Morkūnienė: Visualization, Investigation, Formal analysis. **Laura Jūrienė:** Writing – original draft, Visualization, Validation, Software, Methodology, Investigation. **Petras Rimantas Venskutonis:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of Competing Interest

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

Data availability

Data will be made available on request.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jcou.2024.102890.

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