Extraction of lipophilic components from rowanberry (*Sorbus aucuparia* L.) pomace with supercritical CO_2 and their fractionation at subcritical conditions at low temperatures

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Highlights

- Rowanberry pomace was extracted with supercritical CO_2 and co-solvent ethanol
- Every 1% of added ethanol increased the total extract yield on average by 8.6%
- The extracts were fractionated by reducing separator's pressure and temperature
- Tocopherols, phytosterols and β-carotene were determined in the fractions
- The fractions with the increased concentration of phytochemicals were obtained

Abstract

Rowanberry pomace lipids were extracted with supercritical CO_2 (42.4 MPa, 53 °C) using 3-7% of a co-solvent ethanol and fractionated by reducing 1st separator's (S1) pressure to 7 MPa and cooling to 0, -10 and -20 °C for precipitating 'heavier' fraction (HF). The second separator (S2) was depressurized at ambient temperature for collecting 'lighter' fraction (LF). The yield of LF increased by decreasing S1 temperature and increasing the amount of a co-solvent. The concentration of β-carotene increased in LF by decreasing S1 temperature and increasing co-solvent concentration; at -20 °C it was by 66.7% higher than in the non-fractionated extract. The concentrations of tocopherols and phytosterols were also remarkably higher in LF. In total, 62 compounds were identified in the headspace volatile fraction of LF, benzaldehyde and benzyl alcohol being the most abundant constituents. In

conclusion, fractionation enabled obtaining the fractions with the higher concentration of selected lipophilic rowanberry constituents.

Keywords: *Sorbus aucuparia,* berry pomace, supercritical CO_2, co-solvent, separation, fractions, volatile components

1. Introduction

Pressing fruit juice, besides the main product, generates side-stream residue (called press-cake or pomace), which constitutes approximately 30% of fruit solids and is regarded as a less valued by-product. Therefore, large amounts of pomace are used rather inefficiently, e.g. as animal feed ingredient, for composting or even discarded on the landfills as a waste, thus causing the loss of valuable nutrients and environmental pollution. Consequently, proper valorisation of pomace via their conversion into higher-value products is an important task, which may significantly increase the sustainability of the horticultural and fruit processing sectors. Ideally, the pomace should be upcycled for obtaining the highest added value ingredients and reaching zero waste goal (European Commission, 2020).

Many small fruit species, which are commonly called berries, are particularly rich in polyphenolic antioxidants, natural pigments and various other bioactive compounds. Large fractions of berry constituents remain in the pomace after pressing the juice; in addition, the pomace contains dietary fibre, polyunsaturated fatty acid-rich oil, proteins and various health-beneficial micronutrients (Venskutonis, 2020). Therefore, development of efficient recovery processes of such constituents from the pomace for nutritional and other human uses is an important and challenging task. Considering the heterogeneity of pomace, which consists of pulp, skins, seeds and some stems, different concepts may be used for its processing. Drying and milling is the simplest technology to produce pomace ingredients, while recovery of the selected target groups of compounds as well as their fractionation and purification requires more sophisticated techniques. For instance, during the last few years consecutive extraction of different polarity pomace constituents using supercritical fluid extraction with carbon dioxide (SFE-CO_2), pressurized higher polarity liquids and, in some cases, enzymes was applied to raspberry (Kryževičiute et al., 2016), chokeberry (Grunovaite et al., 2016), black currant (Basegmez et al., 2017), sea-buckthorn (Kitrytė et al., 2017), guelder-rose berry (Kraujalis et al., 2017), lingonberry (Kitrytė, Kavaliauskaitė, et al., 2020), blackberry (Kitrytė, Narkevičiūtė, et al., 2020), cranberry (Tamkutė, Liepuoniūtė, et al., 2020) and rowanberry (Bobinaitė et al., 2020) pomace. This approach enables to recover lipophilic and higher polarity fractions, while the residue contains increased content of insoluble

dietary fibre and proteins. The products obtained may be used as high nutritional valued food, nutraceutical and cosmeceutical ingredients. The lipophilic berry pomace fractions mainly consist of a PUFA-rich triacylglycerols (TAGs) and various micro-constituents like tocopherols, phytosterols and carotenoids, whose health benefits are well-documented; EFSA granted health claims to oleic, linoleic, α-linolenic acids and phytosterols, as contributing to the maintenance of normal blood cholesterol levels, and α-tocopherol (vitamin E) as contributing to the protection of cells from oxidative stress ("Commission Regulation (EU) No 432," 2012).Therefore, there is a great interest for the recovery and application of these lipid-soluble components for food, cosmetics and nutraceuticals. Moreover, considering human applications of the recovered natural components, there is a clear tendency of using green, food and environment friendly technologies as alternatives to conventional extraction methods, particularly those that apply hazardous solvents. From this point of view, SFE-CO_2 has been tested and commercialised for extraction of lipophilic substances from various raw materials, including berry pomace. The main advantages of using CO_2 are its non-toxicity, low price and availability, as well as easy recycling and obtaining solvent-free extracts without energy consuming evaporation procedure. The dissolving ability of CO_2 strongly depends on its pressure and temperature, which may be easily controlled.

In some cases, polar co-solvent, such as ethanol, may be added to SFE-CO_2 flow for adjusting solvent polarity and thereby increasing extraction yield and the recovery rates of medium polarity constituents. In addition, the mild critical temperature (31.1 °C), and oxygen-free medium in SFE-CO_2 processes supports the stability of oxidizable and thermally unstable.

Different anatomical parts of rowanberries have been used in traditional medicines as natural medication for treating inflammatory, bacterial and viral diseases, including tumors, diabetes, neurological, as well as cardiovascular disorders (Sarv et al., 2020). Most of the investigations connected with rowanberry chemical composition and properties have been focused on the polyphenolic antioxidants in these crops (Sarv et al., 2020, 2021), whereas only two studies reported isolation of lipophilic components from rowanberry pomace by SFE-CO_2 (Bobinaitė et al., 2020; Ivakhnov et al., 2019). For instance, Bobinaitė et al. (2020) at optimized for the highest yield conditions (45 MPa, 60 °C, 180 min) obtained 4.8% of lipophilic rowanberry extract; however, the recovery of carotenoids was only up to 49.7% of the amount achieved by the hexane extraction. Other important lipophilic micro-constituents were not reported.

In order to expand our knowledge on the use of SFE-CO_2 both for the recovery of lipophilic rowanberry pomace constituents and their pre-fractionation, this study

was undertaken with an aim to use a co-solvent ethanol for increasing the yield of the extracts and individual groups of constituents and to evaluate the possibilities of increasing their concentration via by using the separator, operating at subcritical parameters at different temperatures. To the best of our knowledge, only one study has applied this concept previously to berry pomace. (Tamkutė, Pukalskas, et al., 2020) had conducted the on-line SFE separation of cranberry pomace lipids, using two separators, while in one of the separators the temperature was kept below zero and pressure below the critical point (subcritical conditions), while the other separator remained under ambient conditions. The authors concluded that the concentration of various lipophilic compounds present in berry pomace could be increased due to the changes of their solubility in liquid CO_2 at freezing temperatures. Considering remarkable compositional differences between the pomace, e.g. the highest yield 4.8% for rowanberries (Bobinaitė et al., 2020) *visa* 11% for cranberries (Tamkutė, Pukalskas, et al., 2020) it was of interest to test this concept for rowanberry pomace at previously optimized extraction conditions for the highest yield and fractionation at subcritical CO_2 parameters. Thus, the hypothesis was that extraction with a co-solvent ethanol at supercritical parameters and fractionation at subcritical pressure and different temperature below zero enables to pre-concentrate the selected groups of lipophilic compounds present in rowanberry pomace. The results obtained may find practical application in the development of the specified ingredients for nutraceuticals.

2. Materials and methods

2.1. Chemicals, solvents and gasses

CO_2 and N_2 were obtained from Gaschema (Jonava, Lithuania), agricultural origin ethanol (96.6%) from MV Group Production (Kaunas, Lithuania), (±)—α-tocopherol, rac- β-tocopherol, (+)- γ -tocopherol, δ -tocopherol, β -carotene, a mixture of 37 fatty acid methyl esters (FAME) were obtained from Sigma-Aldrich (Steinheim, Germany).

2.2. Extraction and fractionation of rowanberry pomace by SFE–CO_2

The fruits of sweet rowanberry cv Sahharnaja were collected in autumn 2021 from Polli Experimental Station, Estonia. The fruits were immediately frozen and stored at -20 °C. Prior to pressing the juice in the low speed juicer Smeg SJF01CREU (Smeg S.p.A, Guastalla, Italy), the fruits were defrosted at ambient temperature. The pomace was lyophilized in an Advantage Plus Benchtop Freeze Dryer (SP Industries, Warminster, PA, USA) during 72 h at 30 μbar, ground in a centrifugal mill Retsch ZM200 (Haan, Germany) using a 0.5 mm sieve and stored in the closed glass jars at room temperature (18 °C).

The SFE was conducted using approximately 20 g of ground pomace in a Helix extractor (Applied Separation, Allentown, PA, USA) equipped with 500 cm^3 extraction cell and two separators, S1 and S2. One micro-metering valve was used for regulating the pressure in the separator S1 and another for keeping the CO_2 flow rate at 2 L/min during the extraction process. In the extraction cell the previously optimized pressure of 42.4 MPa and temperature of 53 °C were maintained by a surrounding heating jacket (Bobinaitė et al., 2020). The first fraction (further called as heavier, HF) was precipitated by reducing the temperature in S1 to 0, −10 and −20 °C, while the pressure was maintained at 7 MPa. The cooling of the separator S1 was achieved by using a thermostatic bath and FT402 FT Immersion Cooler (JULABO GmbH, Seelbach, Germany). The second fraction (further called as lighter, LF), which remained soluble at the liquid CO_2 parameters was collected in the depressurized S2 at room temperature. A co-solvent ethanol (EtOH) was introduced at the concentrations of 3, 5 and 7% and flow rate of 0.228 mL/min. The durability of every extraction was 2 hours. Preliminary extraction results were tested at similar parameters without using separators.

The volume of the consumed CO_2 during extractions was measured in standard litres per min (SL/min) at standard state: P = 100 kPa, T = 20 °C, = 0.0018 g/mL. The separated fractions were kept in glass bottles at −20 °C for the further analysis.

2.3. Analysis of β-carotene

Determination of β-carotene concentration in the extracts was conducted according to Zymone et al. (Zymone et al., 2018) chromatographically by Waters 2695 liquid separation module (Water Corporation, USA) and by UV–vis detector (UV–vis, 2489, Water Corporation, USA) for monitoring the elution of compounds. β-carotene was quantified at 450 nm. Separation was conducted on a RP-C30 column (5 μm, 250 × 4.0 mm, YMC Europe, Dinslaken, Germany) integrated to a C30 guard column (5 μm, 10 × 4.0 mm, YMC Europe, Dinslaken, Germany). The eluent flow rate was 0.65 mL/min and temperature of the column was maintained at 25 °C. The mobile phase consisted of methanol (solvent A) and methyl tert-butyl ether (solvent B). All the samples were injected at 40% B and held for 5 min. The gradient was changed to 83% B in 50 min, and then to 100% B in 5 min and held for 10 min. Finally, the gradient was changed to 40% B in 5 min and held for 10 min.

2.4. Determination of fatty acids and triacylglycerols (TAG)

Fatty acid composition in sweet rowanberry pomace was analyzed by the Sukhija & Palmquist method (Sukhija & Palmquist, 1988) with modifications. Briefly, the lipids of the milled samples were extracted and methylated in one-step procedure using toluene as a solvent. Heptadecanoic acid (C17:0) was used as an internal standard.

Fatty acid methyl esters were analyzed on an Agilent 6890A GC equipped with a split/splitless injector and a flame ionization detector. A fatty acid methyl ester was separated on a quartz capillary column with a liquid phase CP-Sil 88 (100 m × 0.25 mm, liquid phase layer thickness 0.20 μm). The results are expressed in percentages of individual fatty acid contents per total fatty acids.

TAGs were analyzed by the method of Zeb and Murkovic (Zeb & Murkovic, 2010)using the Waters AQCUITY ultra performance liquid chromatography system (Waters Corp., Milford, MA, USA), equipped with hybrid Brucker Daltonics (Bremen, Germany) time-of-flight/quadrupole mass detector (UPLC-Q/TOF), which was controlled by HyStar software (BrukerDaltonic, Bremen, Germany). Separations were conducted by an Acquity BEH, C18 column, 2.1 × 50 mm and at particle size of 1.7 μm (Waters, Ireland). The autosampler and column oven were kept at 20 °C and 40 °C, respectively. An isocratic solvent system consisting of 18% isopropanol in methanol (0.1% acetic acid) and 0.05% of ammonium acetate were used for the elution of the analytes. The flow rate during the elution was 0.4 mL/min and the separation time was 10 min. Fragmentor's potential was 150 V, capillary voltage 4000 V, drying gas temperature of 350 °C and m/z range 200–1000.

2.5. Determination of tocopherols and sterols

The method described by Slavin and Yu (Slavin & Yu, 2012) was employed for saponifying sterol and tocopherol esters for HPLC analysis, which was performed using Waters Aquity UPLC equipped with an auto sampler, solvent delivery system, column manager and Aquity BEH C18 column (50 × 2.1 mm, 1.7 μm (Waters, Milford, MA)The separation procedure started with 30% A (0.1% formic acid in water) and 70% B (0.1% formic acid in acetonitrile). In 1 min. B was increased to 100% kept for 5 min and restored to the initial conditions in 0.5 min . During the procedure, the temperature of the column was kept at 30 °C and the flow rate of eluents was 0.4 mL/min. MAXIS 4 G Q-TOF mass spectrometer (Bruker Daltonics, Bremen, Germany) in APCI positive mode was used for recording the mass spectra at full scan mode in the m/z range from 200 to 1200 at 3 Hz rate. Other parameters were as follows: 2000 V of capillary voltage; 3000 nA of corona current; −500 V of endplate offset; 400 °C of vaporizer temperature; 1.6 bar of nebulizer gas pressure; 8 L/min of drying gas flow rate; and 200 °C of drying gas temperature. The protonated ion peaks $[M+H]^+$ were used for quantification of analysed components, while the single ion chromatograms were extracted with the accuracy of 0.01 m/z.

2.6. Evaluation of volatile aroma compounds

The volatile composition of extracts was analyzed by gas chromatography - time-off-flight mass spectrometry on a GC×GC-TOF/MS LECO Pegasus 4D system, consisting of an Agilent 7890A GC, a GERSTEL Multipurpose Sampler MPS (Gerstel GmbH,

Mulheim an der Ruhr, Germany), coupled with a high-speed TOF/MS detector (LECO, St. Joseph, MI, USA). Headspace solid phase microextraction (HS-SPME) were performed with an MPS-2 autosampler (Gerstel, Mulheim and er Ruhr, Germany) with a Divinylbenzene/ Carboxen/ Polydimethyl-siloxane fiber (50/30 μm, Supelco, Bellefonte, PA, USA) from 100 mg of rowanberry SFE-CO_2 extract which was placed in a 20 mL vial. The vial was equilibrated at 40 °C for 15 min, then fiber was exposed to the headspace of extract during 30 min at 40 °C. Afterwards, the fiber was desorbed in the GC–TOF/MS injector for 3 min.

The column set consisted of a primary column BPX-5 (30 m, 0.25 mm i.d., 0.25 μm film thickness) (SGE Analytical Science, Australia) connected to a secondary column, BPX-50 (1.4 m, 0.10 mm i.d., 0.1 μm film thickness). The primary oven was programmed as follows: 40 °C (1 min) ramped to 220 °C at 5 °C/min (1 min) finally ramped to 300 °C at 20 °C/min (hold 1 min), with a modulator offset temperature of +15 °C. The transfer line temperature was 250 °C, the GC injector port was set at 150 °C then at rate 720 °C/min ramped to 250 °C with desorption time of 5 min. The carrier gas helium was set at 1 mL/min. The TOF/MS acquisition rate was 10 spectra/s, the mass range used for identification was from 30–550 m/z units. Detector's voltage was set at 1550 V and ion source temperature of 250 °C. Data from the GC × GC-TOF/MS system were collected by ChromaTOF software v.4.22 (LECO) after a solvent peak delay of 220 s in a splitless mode for 60 s, a further valve was opened and purge flow was 20 mL/min; mass spectrum assignment was based on matching against Adams, Nist, MainLib, RepLib mass spectral libraries; signal-to-noise threshold was set as 50 with the minimum similarity accepted was 750. The mean values were calculated from quadruplicate injections.

The identification of volatile components was assigned by comparing their Retention Indices (RI) relative to C_7–C_{30} standard *n*-alkanes, obtained on nonpolar BPX-5 column with those provided in literature (Adams, 2017) and by comparing their mass spectra with the data provided by the Nist, MainLib, RepLib and Adams mass spectral libraries. Positive identification was assumed when good match of mass spectrum and RI was achieved.

2.7. Statistical data evaluation

Mean values and standard deviations were calculated using MS Excel 2016. All analyses were carried out in triplicates, unless specified otherwise. Significant differences among means were determined by one-way ANOVA, using the statistical package Stat-graphics 18-X64. Tukey's HSD (honestly significant difference) was used to determine the significant difference among the treatments at $p < 0.05$. Correlation coefficients were calculated between each of the variables.

3. Results and discussion

3.1. The yields of extracts, fractions, and β-carotene

The total yield of the extract is one of the most important process characteristics. Previously reported yields of lipophilic extracts recovered by SFE-CO_2 from rowanberry pomace were comparatively low, up to 4.8%, while the recovery of carotenoids was less than 50% compared with hexane extraction (Bobinaitė et al., 2020). Adding 5% co-solvent EtOH enabled increased carotenoids recovery from cranberry pomace up to 66.25% (Tamkutė, Pukalskas, et al., 2020). Therefore, in our study several concentrations of a co-solvent were applied (3, 5 and 7 %) in order to increase the yield and the recovery of selected phytochemicals. In the preliminary experiments, the yield of extract significantly increased by adding EtOH; from 5.55 (pure CO_2) to 8.88 g/100g (7% EtOH). Consequently, every 1% of added EtOH increased the total yield on average by 8.6%. The addition of polar solvent increases the solubility of higher polarity constituents and may have an effect on the physical properties of the matrix, e.g. by enhancing the diffusion of the solutes from the solid particles.

The yields of the fractions were dependent both on the concentration of a co-solvent and the temperature in S1. In general, the amount of the LF, which was collected in S2, increased by decreasing the temperature in S1 from 0 to -20 °C and by increasing the amount of a co-solvent. For instance, in case of adding 7% EtOH the yield of LF was 1.9-2.1-fold higher than in case of 3% EtOH. The weight of the precipitated in S1 extracts was not measured due to difficulties of its collection; however, it may be assumed that at higher EtOH concentrations and lower temperatures in S1 the ratio of HF/LF decreased. Most likely, the compounds extracted with higher polarity solvent mixture, e.g. CO_2 + 7% EtOH, remained more soluble in it even at low temperature in S1. Somewhat different result was obtained in case of 5% EtOH, particularly during cooling S1 at -10 °C when the amount of LF was slightly higher than in case of -20 °C in S1. The differences in the yields of fractions provide preliminary information that fractionation may be performed by reducing the pressure and changing the temperature in the S1. In addition, the redistribution of extract fractions also depends on the concentration of a co-solvent. It may be reasonably expected that the solubility of some extracted from rowanberry pomace lipophilic constituents may reduce at freezing temperatures, particularly in case of adding EtOH. For instance, a well-known process of 'winterization' is used for the removal of waxes from the crude ethanolic extracts (Baldino et al., 2020). In general, the results obtained suggest that the compounds, which are additionally solubilized by the added EtOH in the solvent mixture at supercritical CO_2 conditions, do not precipitate in the S1 at freezing temperatures and are carried to the S2.

The total extraction yield of lipophilic components is important indicator of SFE-CO_2 efficiency, but this is not the only important process criterion. In case of extracting the ingredients for functional foods and nutraceuticals, the overall recovery of the target bioactive compounds from the raw material as well as their concentration in the extract, may be even more important process characteristics.

The oil-soluble β-carotene is the most common form of carotene in the plants. It is a red-orange pigment, which can be used as a highly prized colorant by the food industry. Therefore, the recovery of β-carotene, which is also a well-known bioactive compound (vitamin A precursor), is an important indicator of extraction effectiveness.

Table 1. The concentration of β-carotene in rowanberry pomace extract fractions and its recovery with the LF

EtOH, %	Separator S1 temperature (°C)	Yield in LF (%)	β-carotene (mg/100 g extract: LF)	β-carotene recovery in S2 with LF (mg/100 g pomace)	β-carotene, (mg/100 g extract: HF)
3	0	1.87 ± 0.02^{ab}	133.1±2.2^{f}	2.5	187.4±0.1^{l}
	-10	3.15 ± 0.10^{cd}	174.1±0.6^{g}	5.5	48.94±0.15^{d}
	-20	3.61 ± 0.38^{de}	213.3±1.6^{l}	7.7	52.38±0.09^{e}
5	0	2.92 ± 0.10^{bcd}	145.1±1.4^{i}	4.2	131.5±0.2^{f}
	-10	5.53 ± 0.17^{fgh}	219.0±0.8^{n}	12.1	17.82±0.10^{a}
	-20	4.51 ± 0.27^{ef}	236.3±1.2^{jk}	10.7	31.39±0.18^{b}
7	0	3.84 ± 0.37^{de}	186.2±1.5^{m}	7.2	180.3±0.1^{k}
	-10	6.00 ± 0.42^{gh}	178.2±1.0^{o}	10.7	nd
	-20	7.75 ± 0.25^{ij}	238.1±0.6^{o}	18.5	37.41±0.28^{c}

HF – heavier fraction; LF– lighter fraction. nd not determined; a,b Different letters within the same column indicate statistical differences (one-way ANOVA, $P < 0.05$).

The content of β-carotene in hexane extract of the rowanberry pomace was analyzed by UHPLC-DAD-MS/MS. The results revealed that β-carotene content in sweet rowanberry *cv* Sahharnaja pomace extract was 391.9 mg/100 g, while (Bobinaitė et al., 2020) reported 629.1 mg/100 g in the rowanberry pomace from the mixed cultivars.

In the current study, β-carotene content was analysed in the SFE-CO_2 lipophilic extracts separated by fractionation. Preliminary experiments (no fractionation) showed that adding EtOH increased the recovery of β-carotene, approx. by 4.2% for 1% of the added co-solvent. However, due to the increased total extract yield and dilution of β-carotene with other constituents, its concentration in the extracts reduced from 175.7±0.8 mg/100 g (0% EtOH) to 168.7±0.8, 166.1±1.9 and 142.8±2.5 mg/100 g with 3, 5 and 7% of EtOH, respectively.

In the case of fractionation, both the decrease of temperature in S1 as well as the increase of co-solvent concentration increased the concentration of β-carotene in S2 (LF) and decreased in S1 (HF). Thus, the concentration of β-carotene in LF obtained at -20 °C was by 66.7% higher than its concentration in the non-fractionated extract. Moreover, the recovery of β-carotene with LF was higher at lower temperature in S1. Therefore, the highest β-carotene recovery of 60.8% as compared with hexane extract was found in LF at -20 °C and with maximum co-solvent concentration (7%). These results are consistent to the previous study which demonstrated that the increase of a co-solvent EtOH concentration can remarkably increase the solubility of β-carotene in SFE-CO_2 (Ahmadkelayeh & Hawboldt, 2020). Previously reported distribution of β-carotene extracted from cranberry pomace by SFE-CO_2 with 0 and 5% EtOH in the LF and HF using similar fractionation parameters was not so evident (Tamkutė, Pukalskas, et al., 2020). However, the concentration of β-carotene in rowanberry pomace extracts obtained in our study was more than 200 times higher than in cranberry extracts and therefore the fractionation results may not be directly compared because remarkable differences in the concentration may have a crucial effect on the solubility at various temperatures and co-solvent concentrations.

3.2. Composition of fatty acids and triacylglycerols (TAGs)

The preliminary determination of fatty acids revealed that the main fatty acids in pomace oil of sweet rowanberry pomace were linoleic (57.33±1.36%), oleic (22.36±0.57%) and palmitic (9.98±0.52%). Other fatty acids exceeding 1% in the total content were stearic (1.92±0.25%), lignoseric (1.46±0.05%) and behenic (1.39±0.12%) acid. Therefore, the TAGs of pomace composed mainly of these linoleic (L), oleic (O), palmitic (P), linolenic (Ln) and stearic (S) acids. The previous studies reported that in most TAG molecules the fatty acids in the sn1 and sn3-position are different but one of these may be similar to fatty acid allocated in the sn2-position (Mu & Høy, 2004). The composition of rowanberry TAGs agrees with this assumption (Table 2). The majority of identified in rowanberry oil TAGs contain quantitatively major linoleic acid in the sn2-position, while four of TAGs also contain linoleic acid in the sn1 or sn3 position. The major TAGs in rowanberry pomace oil composed of

unsaturated LLL and OLL; their content was 34.93-36.13%, 26.52-27.87%, respectively (Table 2). The second quantitatively largest TAGs, SLL and PLL constituted 11.92-12.82% and 10.43-11.65%, respectively. The TAGs containing all three different fatty acids, including saturated, mono and polyunsaturated were present at 3.09-6.49%, while most highly saturated (PLS) fatty acids were minor TAGs.

In general, the effect of a co-solvent on TAGs composition was negligible, although statistical data evaluation indicates on some increase in the contents of LLLn, SLO, SLL and PLO and decrease of LLL when the co-solvent concentration increased.

The composition of TAGs in LF fractions obtained at different temperatures in S1 was also quite similar. Some redistribution of TAGs may be expected due to the differences in melting points between more saturated and more unsaturated TAGs, however, it seems that the applied in S1 temperature does not have significant effect on the solubility of TAGs in subcritical (liquid) CO_2. According to a previous study (Tamkutė, Pukalskas, et al., 2020), the differences in percentage composition of cranberry pomace TAGs in LF and HF were also not significant. To the best of our knowledge, this is the first report on TAG composition of rowanberry oil.

3.3. Tocopherols and phytosterols

The plant tocopherols are lipophilic antioxidants, which can protect the PUFA-s from peroxidation (Ali et al., 2022). In human nutrition, the daily administration of α-tocopherol (vitamin E) is essential, as it is the most effective lipophilic antioxidant in the human organism.

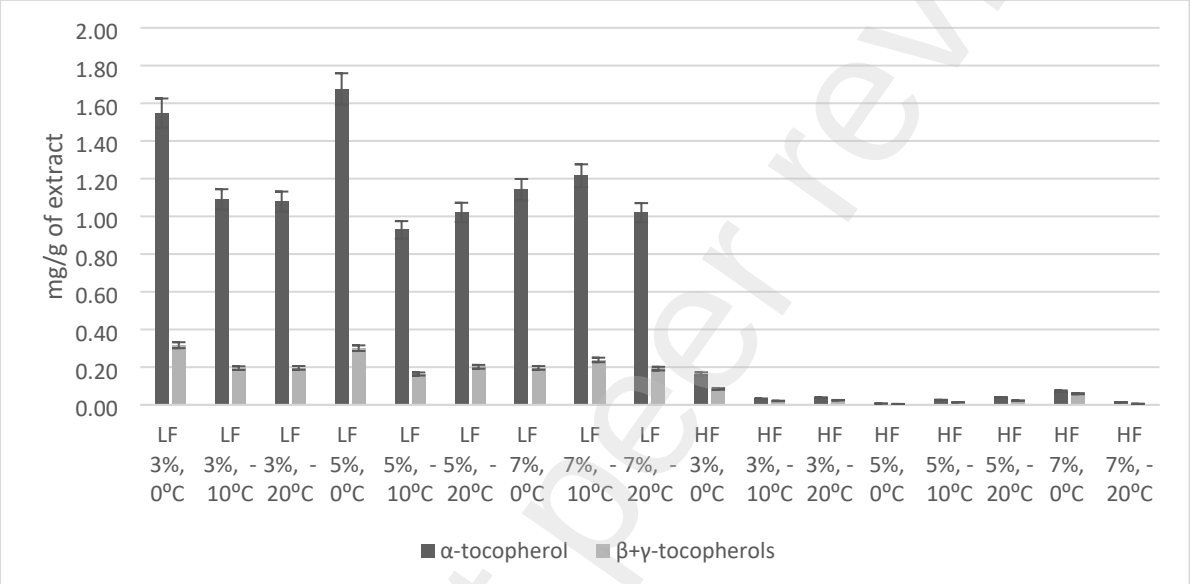
Three tocopherols (α, β and γ) and three phytosterols were identified and quantified in the total extracts and both fractions, LF and HF, while the concentration of α-tocopherol was up to 5.5- fold higher than β+γ-tocopherol at the same conditions. Preliminary evaluation (without fractionation) showed that adding 5 and 7% of ethanol significantly increased the total recovery of tocopherols from the rowanberry pomace. The highest recovery was obtained in case of adding 5% of ethanol, which agrees with the results of Tamkutė et al. (2020) and (Kraujalis & Venskutonis, 2013) on SFE-CO_2 of cranberry pomace and amaranth, respectively. Thus, an average increase in the recovery of α and β+γ-tocopherols for each added 1% of ethanol was 3.5 and 0.84 μg/g of pomace, respectively. However, the effect of ethanol on the concentration of tocopherols in the extracts was not so evident due to the increase in the total yield and consequent dilution of tocopherols with other extracted substances.

Table 2. Composition of triaglycerols (TAGs, %) of rowanberry pomace oil separated in S2 (LF)

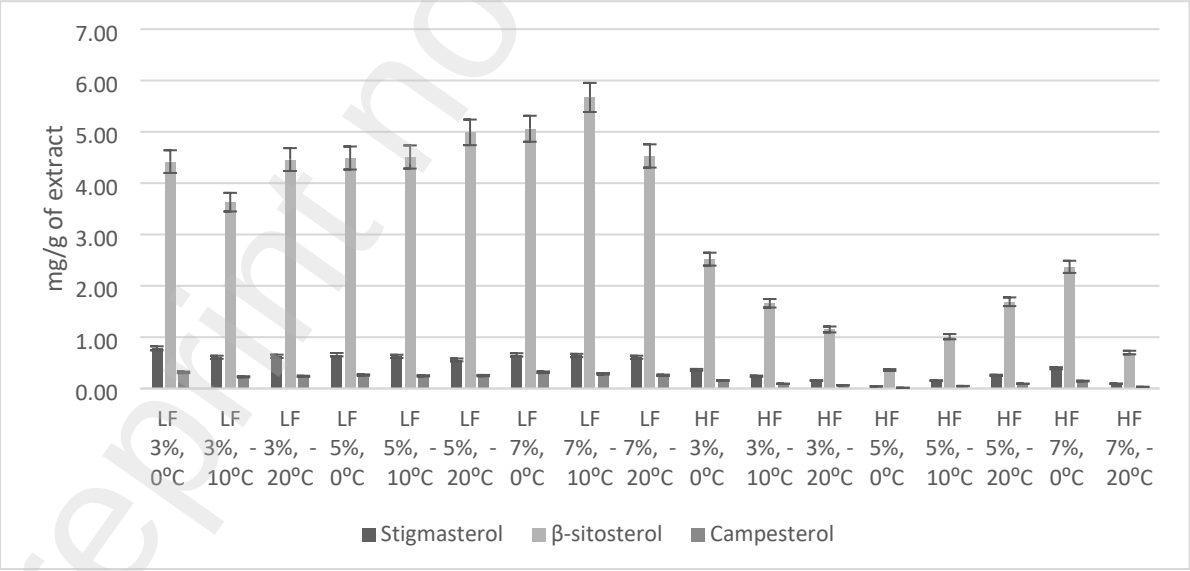
No	TAG	0% EtOH	3% EtOH	5% EtOH	7% EtOH	Light fraction (LF)								
						0°C			-10°C			-20°C		
						3 % EtOH	5 % EtOH	7 % EtOH	3 % EtOH	5 % EtOH	7 % EtOH	3 % EtOH	5 % EtOH	7 % EtOH
1	LLLn	1.76±0.01a	1.59±0.05a	1.64±0.03a	1.65±0.03a	1.74±0.02a	1.62±0.08a	1.74±0.06a	1.71±0.08a	1.62±0.11a	1.69±0.03a	1.63±0.01a	1.62±0.08a	1.57±0.01a
2	SLO	3.09±0.06a	3.18±0.01ab	3.35±0.14ab	3.66±0.21b	3.24±0.12ab	3.30±0.05ab	3.49±0.26ab	3.45±0.01ab	3.38±0.13ab	3.37±0.11ab	3.36±0.15ab	3.45±0.06ab	3.43±0.06ab
3	PLLn	1.04±0.02d	0.92±0.00cd	0.88±0.05bcd	0.92±0.09cd	0.92±0.06cd	0.86±0.04abc	0.78±0.02abc	0.83±0.04abc	0.83±0.01abc	0.74±0.02ab	0.79±0.04abc	0.83±0.04abc	0.70±0.03a
4	PLS	1.74±0.02ab	1.80±0.02ab	1.67±0.16a	2.15±0.29b	1.70±0.19ab	1.59±0.05a	1.67±0.06a	1.83±0.06ab	1.55±0.04a	1.75±0.05ab	1.71±0.14ab	1.70±0.05ab	1.82±0.01ab
5	OLL	26.77±0.50ab	27.00±0.08abc	27.44±0.11abc	27.09±0.30abc	26.52±0.50a	27.05±0.00abc	27.35±0.43abc	27.17±0.02abc	27.87±0.00c	27.18±0.15abc	27.76±0.09bc	27.59±0.27bc	27.08±0.12abc
6	PLL	11.65±0.09d	10.70±0.04ab	10.71±0.02ab	10.64±0.25ab	11.42±0.08cd	11.24±0.40bcd	10.67±0.02ab	11.02±0.10abcd	10.79±0.09abc	10.60±0.06ab	10.67±0.33ab	10.63±0.02ab	10.43±0.16a
7	LLL	35.26±0.27a	36.13±0.25a	35.52±0.60a	34.93±0.16a	35.57±0.22a	35.57±0.64a	35.68±0.13a	35.01±0.43a	34.88±0.16a	36.01±0.00a	35.62±0.38a	35.28±0.32a	36.12±0.42a
8	SLL	11.92±0.09a	12.54±0.15b	12.55±0.04b	12.62±0.22b	12.54±0.13b	12.35±0.32ab	12.48±0.06b	12.62±0.15b	12.82±0.00b	12.57±0.07b	12.38±0.13ab	12.79±0.06b	12.72±0.04b
9	PLO	6.49±0.08b	6.13±0.11ab	6.22±0.05ab	6.35±0.02ab	6.35±0.08ab	6.43±0.03ab	6.13±0.03ab	6.36±0.14ab	6.25±0.14ab	6.07±0.05a	6.06±0.15a	6.11±0.07a	6.12±0.12a

L – linoleic acid; Ln – linolenic acid; O – oleic acid; P – palmitic acid; S – stearic acid. [a,b] Different letters within the same row indicate statistical differences (one-way ANOVA, $P < 0.05$).

It is evident that the major part of tocopherols was transferred to S2 (Fig. 1a, LF); for instance, the concentration of α-tocopherol in LF was at least 10-fold higher than in HF. The highest concentration of α-tocopherol (1675 ± 136 μg/g) and β+γ-tocopherol (300.8 ± 2.8 μg/g) was found in LF obtained in case of using 5% EtOH and at 0°C in S1;it was by 39% higher than in the total extract isolated at similar extraction parameters. Somewhat similar tendencies were observed in case of 3% EtOH, while the differences in the concentrations of tocopherols in LF at different temperatures in S1 were not so evident. Similar results were reported for cranberry pomace in which the results were expressed in chromatogram peak area (Tamkutė at al., 2020).



a)



b)

Figure 1. The concentration of tocopherols a) and phytosterols b) (mg/g of extract) in the fractionated SFE-CO_2 extracts; HF – heavier fraction; LF– lighter fraction.

Phytosterols or plant sterols involve more than 250 various sterols, while the most abundant are sitosterol, stigmasterol and campesterol. Phytosterols, as precursors of plant growth factors, play an essential role in the function and structure of cell membranes, similarly to cholesterol in mammalian cells (Piironen et al., 2000). For patients with elevated blood cholesterol levels the suggested daily dose of plant sterols would be 2 g as a food supplement (Nattagh-Eshtivani et al., 2022). In the previous study, where the rowanberry cuticular wax was extracted by chloroform and a mixture of hexane/ethyl acetate, the β-sitosterol content was 2.91 mg/g of extract (Klavins & Klavins, 2020).

In the current study, where the SFE-CO_2 extraction of the rowanberry pomace was carried out with EtOH as a co-solvent with fractionation, the concentrations of β-sitosterol in LF ranged between 3.63 mg/g to 5.67 mg/g, being many times higher than the contents in the HF, 0.36 mg/g to 2.58 mg/g, respectively (Fig.1b). The concentrations of stigmasterol in LF and HF were 0.56 - 0.78 and 0.09-0.39 mg/g, respectively, while the concentrations of campesterol were 0.23 – 0.31 and 0.03 – 0.16 mg/g, respectively. Consequently, the concentration of phytosterols was always higher in LF than HF; however, the results obtained did not reveal any unambiguous tendencies regarding the effects of a co-solvent and temperature in the S1.

3.4. Volatile constituents

Totally, 62 volatile compounds were identified in the headspace of different sweet rowanberry cv Sahharnaja pomace SFE-CO_2 extracts, which accounted 88.4-96.9% area of the total area of the identified peaks. The constituents that were detected at least in three rowanberry extract samples are provided in Table 3, which shows that the quantitative composition of volatiles is rather complex consisting of aromatics, alcohols, acids, aldehydes, lactones, esters, terpenes, *n*-alkanes, and others class of compounds. Benzaldehyde and benzyl alcohol were the most abundant compounds released in the headspace of SFE-CO_2 extract; these results agree with the previously reported data on volatile composition of rowanberry fruits (Doležal et al., 2001, 2003; Houlberg et al., 2000). 2-Pentanone, benzaldehyde, methyl butyrate were found to be the major volatiles in aroma extracts of rowanberry fruits (Doležal et al., 2003), while benzaldehyde, (E)-2-hexenal and benzyl alcohol were selected by AEDA as principal aroma-active components (Doležal et al., 2001). In another study, benzaldehyde, benzyl alcohol and acetic acid were found in high amounts in alcohol extracts of dried rowanberries, whereas terpenoids and acids were determined at lower concentrations (Houlberg et al., 2000).

The content of some other components exceeded 1%, e.g. the content of 2-oxo-2-phenylacetonitrile (benzoyl cyanide) was 0.06 – 3.27%, phenyl ethyl alcohol 1.09 – 2.52%, benzoic acid 0.19 – 2.57%, diethyl hydroxybutanoate 0.17 – 12.74%, furfural 0.19 – 2.06%, butyrolactone 0.30 – 1.57% and etc. (Table 3). In general, volatile profile of

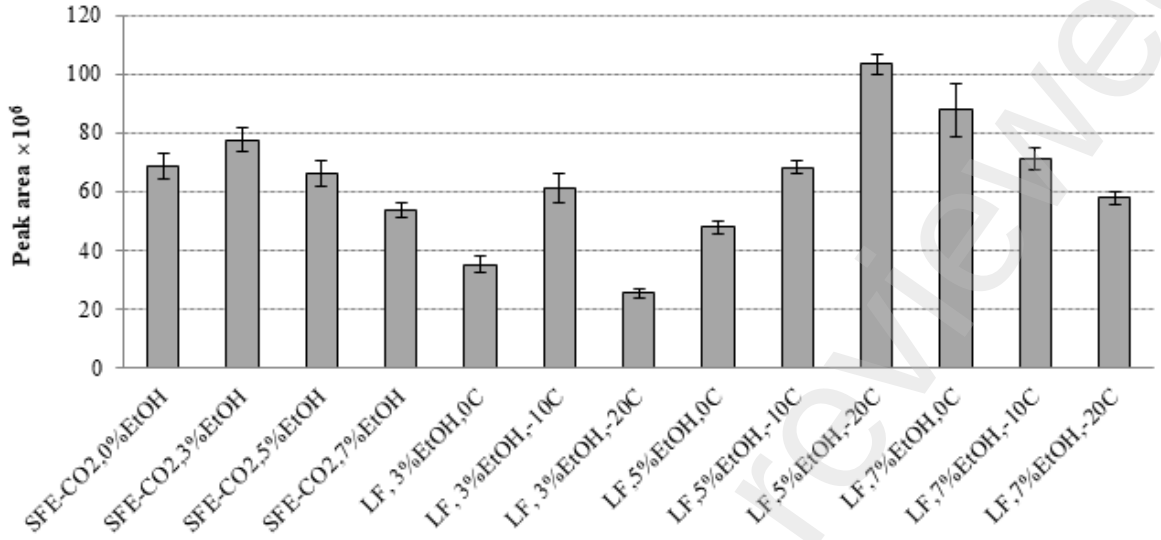


Figure 2. Benzaldehyde peak area in the headspace of the total rowanberry extracts (T) and its fraction (LF) at different EtOH concentrations (%) and separation temperatures in S1 (°C)

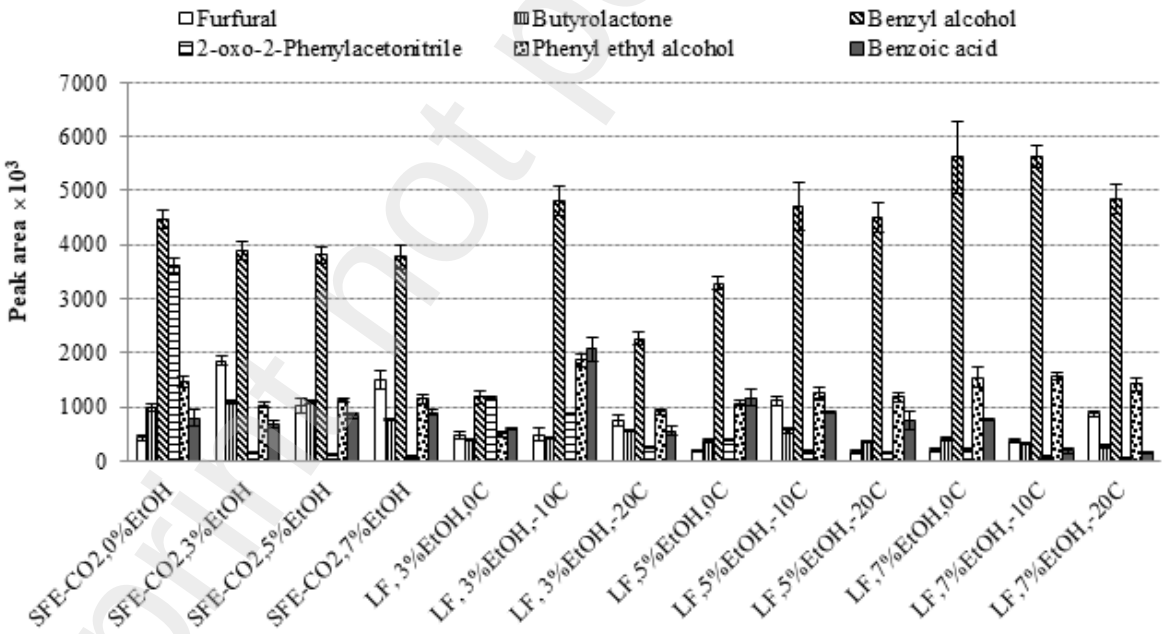


Figure 3. The major volatiles` peak area in the headspace of the total rowanberry extracts (T) and its fraction (LF) at different EtOH concentrations (%) and separation temperatures in S1 (° C).

total extracts and their LFs was quite similar although some variations were observed for the products obtained at different separation temperatures. For example, the percentage content of major compound benzaldehyde in total SFE-CO_2

Table 3. Volatile compounds detected in the total and light fractions of SFE-CO_2 rowanberry pomace extracts, peak area percentage (%)

No#	Compound A.*	RI-E	RI-L	Total				Light fractions								
				SFE-CO_2 + EtOH,%				SFE-CO_2 + 3% EtOH			SFE-CO_2 + 5% EtOH			SFE-CO_2 + 7% EtOH		
				0	3	5	7	0	-10	-20	0	-10	-20	0	-10	-20
1	Furfural	832	828	0.38±0.04	1.98±0.07	1.17±0.11	1.94±0.18	1.07±0.05	0.60±0.09	2.06±0.20	0.30±0.05	1.28±0.06	0.14±0.01	0.19±0.02	0.39±0.04	1.06±0.09
2	2-Methyl butanoic acid	846	841	0.16±0.01	0.09±0.01	0.12±0.01	0.15±0.01		0.12±0.01			0.11±0.00	0.09±0.00	0.10±0.01	0.09±0.00	
3	Heptanal	905	901	0.33±0.01				0.36±0.01			0.12±0.02					
4	Butyrolactone	938	941	0.88±0.02	1.16±0.06	1.27±0.04	1.00±0.05	0.88±0.04	0.51±0.02	1.57±0.05	0.56±0.04	0.65±0.02	0.30±0.00	0.37±0.02	0.32±0.02	0.32±0.04
5	*(2E)*-Heptenal	949	945	0.88±0.02		0.74±0.00	0.10±0.01	0.58±0.07	0.16±0.02		0.15±0.01			0.09±0.00		
6	γ-Valerolactone	951	948			0.14±0.02					0.17±0.01	0.14±0.01				
7	Ethyl cyanacetate	960	959				0.07±0.01							0.13±0.01	tr	
8	Benzaldehyde	961	960	71.60±1.32	82.89±1.73	77.39±0.49	69.66±2.32	78.08±0.71	75.90±1.24	70.73±0.90	72.92±2.31	78.44±0.79	87.75±0.92	79.99±0.82	72.18±1.99	69.53±0.96
9	δ-Valerolactone	965	965		0.17±0.02	0.21±0.01	0.17±0.01	0.14±0.00	0.12±0.01			0.09±0.01		0.07±0.01	0.07±0.01	0.07±0.00
10	6-Methyl-5-hepten-2-one	985	984	1.80±0.03		0.13±0.03					0.14±0.00	0.30±0.01	0.31±0.02	0.32±0.01		
11	*n*-Octanal	999	998	0.04±0.01											0.08±0.01	0.10±0.00
12	Hexanoic acid	1000	998	0.71±0.04	0.13±0.01	0.15±0.00	0.17±0.01	1.83±0.02	0.36±0.01	0.48±0.02	0.70±0.03	0.50±0.06	0.16±0.01		0.22±0.01	0.18±0.01
13	Ethyl oxalate	1002	998		0.13±0.02	0.37±0.02	1.29±0.05		0.20±0.01		0.15±0.09		0.11±0.00		1.83±0.06	1.36±0.06
14	Ethyl hexanoate	1005	1003		0.05±0.01	0.06±0.00	0.08±0.01	0.07±0.00	tr		0.10±0.01	0.12±0.00	tr	0.08±0.00	0.09±0.00	tr
15	*(2E,4E)*-Heptadienal	1009	1007	0.07±0.00	0.05±0.00		0.09±0.01	0.12±0.01	0.21±0.03			0.07±0.00	0.06±0.00	0.08±0.01	0.08±0.00	0.07±0.00
16	2H-Pyran-2,6(3H)-dione	1011	-		0.25±0.01	0.28±0.01	0.39±0.02		0.30±0.01	0.34±0.02	0.24±0.04	0.30±0.01	0.17±0.01	0.24±0.03	0.44±0.02	0.42±0.02
17	2,2,6-trimethylcyclo-hexanone	1033	1027		0.35±0.02	0.38±0.09	0.42±0.03	0.20±0.01	0.18±0.02	0.46±0.03	0.25±0.01	0.15±0.00	0.12±0.04	0.19±0.02	0.26±0.05	0.16±0.03
18	Benzyl alcohol	1040	1031	4.02±0.10	4.15±0.08	4.47±0.16	4.90±0.25	2.61±0.03	5.99±0.19	6.35±0.03	5.01±0.12	5.39±0.28	3.83±0.08	5.12±0.22	5.72±0.09	5.83±0.23
19	Pantolactone	1042	1032			0.08±0.02	0.07±0.02					0.08±0.00	tr	0.05±0.01	0.07±0.00	0.08±0.00
20	Benzene acetaldehyde	1042	1036		0.05±0.00	0.06±0.00	0.09±0.01		0.13±0.01			0.08±0.00	0.05±0.00	0.05±0.00	0.09±0.01	0.08±0.00
21	3-Methyl-2-cyclohexen-1-one	1049	1046	0.95±0.03	0.21±0.01	0.24±0.01	0.37±0.02	0.28±0.02	0.17±0.01	0.25±0.03	0.19±0.01	0.16±0.01	0.10±0.03	0.16±0.01	0.18±0.01	0.12±0.01
22	*(2E)*-Octen-1-al	1052	1049	0.93±0.02	0.13±0.01	0.15±0.00	0.28±0.04	0.33±0.02	0.14±0.01	0.26±0.02				0.14±0.01	0.15±0.01	0.11±0.01

23	Ethyl levulinate	1078	1070				0.25±0.04					0.07±0.01			0.12±0.01	0.15±0.00
24	Ethyl diethoxyacetate	1096	1092			0.20±0.01	0.07±0.01				0.41±0.02	0.54±0.03	0.25±0.01		0.17±0.01	0.15±0.01
25	2-oxo-2-Phenylacetonitrile	1104	1095	3.27±0.08	0.17±0.01	0.14±0.01	0.11±0.01	2.60±0.16	1.09±0.06	0.69±0.05	0.58±0.03	0.19±0.01	0.13±0.00	0.19±0.01	0.08±0.00	0.06±0.00
26	*n*-Nonanal	1108	1100	0.14±0.01	0.07±0.00	0.08±0.00	0.13±0.01	0.13±0.01	0.14±0.01	0.15±0.01	0.17±0.01	0.07±0.00	0.06±0.00	0.09±0.00	0.11±0.00	
27	Phenyl ethyl alcohol	1119	1107	1.33±0.02	1.11±0.03	1.31±0.06	1.49±0.10	1.09±0.01	2.33±0.06	2.52±0.02	1.63±0.04	1.43±0.07	1.00±0.02	1.41±0.07	1.58±0.04	1.71±0.10
28	2-Ethyl hexanoic acid	1132	1119					0.30±0.02	0.14±0.00	0.28±0.00		0.19±0.02	0.08±0.01			
29	Benzyl acetate	1166	1157	0.07±0.00	0.12±0.00	0.14±0.01	0.17±0.01		0.11±0.01	0.19±0.01	0.12±0.00	0.14±0.00	0.12±0.00	0.13±0.01	0.18±0.01	0.19±0.00
30	Ethyl benzoate	1182	1169	tr	0.07±0.00	0.12±0.01	0.17±0.01	0.07±0.01	tr	0.08±0.00	0.08±0.01	0.13±0.00	0.05±0.00	0.09±0.01	0.20±0.00	0.27±0.01
31	Diethyl succinate	1190	1176				0.10±0.00					tr			0.21±0.00	0.32±0.02
32	Octanoic acid	1200	1190					0.10±0.02			tr	0.05±0.01		tr		
33	Benzoic acid	1202	1197	0.71±0.13	0.73±0.08	0.98±0.08	1.17±0.04	1.32±0.17	2.57±0.05	1.55±0.22	1.78±0.21	1.03±0.03	0.62±0.14	0.70±0.05	0.19±0.04	0.19±0.01
34	β-Cyclocitral	1230	1217	0.30±0.00	0.06±0.01	0.07±0.01	0.10±0.02		0.11±0.00		0.09±0.02	0.05±0.01	0.05±0.01	0.06±0.01	0.08±0.01	0.09±0.00
35	Ethyl phenylacetate	1255	1243			0.05±0.00	0.08±0.01					tr			0.11±0.00	0.16±0.01
36	Diethyl hydroxybutanoate	1256	1244		0.17±0.01	0.60±0.02	2.45±0.19		0.52±0.03	0.70±0.05	0.45±0.04	1.48±0.04	0.38±0.04		6.94±0.08	12.74±0.58
37	2-Phenyl ethyl acetate	1257	1254	tr	0.07±0.01	0.07±0.00	0.09±0.01		0.12±0.01	0.15±0.00	0.13±0.01	0.08±0.00	0.05±0.00	0.07±0.00	0.08±0.00	0.08±0.01
38	Nonanoic acid	1272	1267					0.21±0.01			0.13±0.00			0.09±0.01		
39	α-Cyanobenzyl alcohol	1312	1301	0.14±0.02	0.07±0.00	0.10±0.01	0.21±0.02	0.82±0.12	0.89±0.03	0.64±0.05	0.63±0.10	0.36±0.05	0.09±0.02	0.15±0.02	0.20±0.02	0.30±0.03
40	Geranyl acetone	1453	1453	0.17±0.02		0.06±0.00	0.09±0.01	0.15±0.01	0.15±0.01	0.07±0.02	0.11±0.01		0.05±0.00		0.07±0.01	0.09±0.01
41	*(E)*-β-Ionone	1488	1487	0.30±0.04	0.06±0.00	0.08±0.00	0.11±0.01	0.18±0.01	0.18±0.01	0.25±0.01	0.14±0.01	0.07±0.00	0.07±0.01	0.05±0.00	0.08±0.00	0.11±0.01
42	Dihydroactinidiolide	1551	1539	0.51±0.03	0.18±0.02	0.21±0.01	0.27±0.03	0.73±0.06	0.58±0.03	0.76±0.05	0.49±0.04	0.22±0.01	0.18±0.02	0.15±0.01	0.20±0.01	0.25±0.02
	Total identified, %			92.06	94.67	91.62	88.38	94.59	94.36	90.54	90.70	94.90	96.43	94.78	96.92	96.47

[#] Compounds are listed in order of their elution from nonpolar BPX-5 MS capillary column.

* 3-Methyl butanoic acid, 2-heptanone, pentanoic acid, α-pinene, 4-methyl-pent-2-enolide, 1-octen-3-ol, myrcene, 2-pentyl furan, decane, *p*-cymene, limonene, γ-terpinene, 5,6-dihydro-2H-pyran-2-one, γ-hexalactone, *(Z)*-linalool oxide, *(E,E)*-3,5-octadien-2-one, γ-ethoxy butyrolactone, dodecane, ethyl octanoate and β-Ionone epoxide were detected only in one or two samples at the percentage concentration 0.04 - 0.80%.

[A] Identified on the basis of GC–TOF/MS spectra based on comparison with Adams, Nist, PubChem and Chemspider databases and calculated RI.

RI-E, Retention indices calculated against C_7–C_{30} *n*-alkanes on nonpolar BPX-5 MS column.

RI-L, Retention indices on nonpolar DB-5 column reported in literature(Adams, 2007)or Nist (https://webbook.nist.go), PubChem (https://pubchem.ncbi.nlm.nih.gov) and Chemspider (https://www.chemspider.com) databases.

extracts was from 69.66±2.32% (0% EtOH) to 82.89±1.73% (3% EtOH). In the case of LFs the contents varied from 69.53±0.96 (LF, 3% EtOH, -20° C) to 87.75 ± 0.92% (LF, 5% EtOH, -20° C) (Table 3). Although the highest content of benzaldehyde was achieved at -20° C with 5% EtOH, at lower EtOH concentrations the highest levels of benzaldehyde were obtained at the highest temperature (0°C) used in current study. The highest quantities of the other major volatile compounds, such as butyrolactone, furfural, benzyl alcohol and phenyl ethyl alcohol were identified at -20°C with 3% EtOH, however, for benzoic acid and 2-oxo-2-phenylacetonitrile the most suitable temperatures with the same amount of EtOH were -10°C and 0 °C, respectively.

Volatile compounds in HFs were not analyzed due to the insufficient amount of these fractions. However, based on the previously reported data for cranberry extracts, which demonstrated that their HFs were almost empty of volatiles, it may be reasonably assumed that the utterly major part of rowanberry volatile compounds will be transferred to S2 (LF).

Figure 2 illustrates the concentration of major aromatic compound benzaldehyde, which was released into headspace of rowanberry pomace SFE-CO_2 extracts and was expressed in peak area units (au). Peak area provides more comprehensive information about extraction efficiency as long as it is directly related to the absolute concentration of the released volatiles and could be closely related and to the sensory properties of rowanberry extracts obtained at different extraction conditions. Thus, the amounts of benzaldehyde in different rowanberry SFE-CO_2 extracts were significantly different and varied from (25.18 ± 1.28) × 10^6 au (LF, 3% EtOH, -20 °C) to (103.31 ± 1.28) × 10^6 au (LF, 5% EtOH, -20 °C) (Figure 2). It may be assumed that benzaldehyde as a major aroma compound would play an important role in the overall rowanberry extract aroma; it possesses "almond, burnt sugar (Acree & Arnold, 2004) "sharp, sweet, bitter almond, cherry" and "almond, fruity, powdery, nutty, cherry, maraschino cherry" odour notes (*The Good Scents Company Information System*, 2022). Naturally benzaldehyde occurs in various plant origin foods, such as almonds, apricots, cherry kernel, apples, black tea, Chardonnay wine, etc. (Welke et al., 2014). Benzaldehyde is commonly employed to confer almond flavor to foods and scented products; also, it is used in cosmetic. As intended used in food, cosmetics, pharmaceuticals, or soap, benzaldehyde is "generally regarded as safe" (GRAS) by the US FDA and FEMA. The absolute amounts of other quantitatively important volatile compounds are summarized in Figure 3.

Benzyl alcohol, as the second major volatile compound, is an aromatic alcohol, which has been characterized as possessing "sweet, flower"(Acree & Arnold, 2004),"berry, cherry, grapefruit, citrus, walnut" "floral, rose, phenolic, balsamic, sweet, fruity and chemical" odour notes" (*The Good Scents Company Information System*, 2022). The percentage content of benzyl alcohol in different rowanberry SFE-CO_2 extract was from 2.61±0.03% (LF, 3% EtOH, 0 °C) to 6.35±0.03% (LF, 3% EtOH, -20 °C) (Table 3); the absolute amount varied from (1.17 ± 0.11) × 10^6 au (LF, 3%EtOH, 0 °C) to (5.61 ± 0.67) × 10^6 au (LF, 7%EtOH, 0 °C) (Figure 3).

The percentage distribution of benzoic acid was from 0.19 to 2.57% (Table 3); the absolute amount varied from (154.4 ± 8.6) × 10^3 au (LF, 7% EtOH, -20 °C) to (2.06 ± 0.22) × 10^6 au (LF, 3% EtOH, -10 °C) (Figure 2). Naturally occurring benzoic acid is strong antimicrobial agent; it has a balsamic, sweet, honey-like aroma and was reported as major aroma contributors of cranberry vines, juices and cranberry pomace SFE-CO_2 extracts (Tamkutė, Pukalskas, et al., 2020) .

Benzyl alcohol, benzaldehyde and benzoic acid are used in a wide variety of cosmetics formulations as preservatives and fragrance ingredients. The group of benzyl derivatives was reaffirmed as generally recognized as safe (GRAS) by the 'Expert Panel of the Flavour and Extract Manufacturers' (FEMA), and the evidence of safety is supported by the fact that the intake of benzyl derivatives as natural components of traditional foods is larger than their intake in case of intentional adding as flavouring substances (Adams et al., 2005). It may be concluded that *S. aucuparia* pomace SFE-CO_2 extracts and their fractions might be a potential natural flavourings providing aroma notes of benzaldehyde, benzyl alcohol and some other natural phytochemicals.

4. Conclusions

The simultaneous separation of rowanberry pomace lipophilic compounds isolated by SFE-CO_2 with 3, 5 and 7% of a co-solvent (EtOH) enabled to obtain two lipophilic products, conditionally named as 'heavier' (HF), collected in the 1st separator (S1) at 7 MPa and 0, -10 and -20 °C, and 'lighter' (LF), collected in the depressurized 2nd separator (S2) and ambient temperature, fractions with different concentrations of selected micro constituents. Every 1% of added co-solvent EtOH increased the total yield of extract on average by 8.6%, while the yield of LF increased by decreasing the temperature in S1 from 0 to -20 °C and by increasing the amount of a co-solvent. EtOH increased the recovery of β-carotene, approx. by 4.2% for 1% of the added co-solvent; however, due to the increased total extract yield and dilution of β-carotene with other constituents, its concentration in the extracts reduced from 175.7±0.8 (0% EtOH) to 142.8±2.5 mg/100 g (7% EtOH). The decrease of S1 temperature as well as the increase of a co-solvent concentration increased the concentration of β-carotene in LF and decreased in HF; in LF obtained at -20 °C it was by 66.7% higher than in the non-fractionated extract. The concentrations of phytosterols and tocophereols in LF were also remarkably higher than in HF; however, any clear dependence of the effect of a co-solvent concentration and S1 temperature on the compound concentration was not observed . Among 62 identified volatile compounds in the total rowanberry pomace SFE-CO_2 extracts and their LF, the most abundant were benzaldehyde and benzyl alcohol, constituting up to 87.75 ± 0.92% (LF, 5% EtOH, -20°C) and up to 6.35±0.03% (LF, 3% EtOH, -20 °C), respectively. The effect of a co-solvent and fractionation on quantitatively major lipophilic constituents, triacylglycerols and fatty acids, was negligible; the major TAGs of rowanberry

pomace were composed of the major fatty acids – oleic and linoleic LLL and OLL forming 34.93-36.13%, 26.52-27.87% of TAGs, respectively.

In general, the results obtained in this study have proved the hypothesis about the possibility to pre-concentrate the selected groups of lipophilic compounds present in rowanberry pomace by the precipitation of the insoluble in the subcritical CO_2 fraction in the separator installed after the main extractor and cooled below 0°C. Moreover, the use of a co-solvent ethanol may increase the total yield of the extracts and recovery of β-carotene. These findings enable to upcycle the fruit pomace in order to obtain the beneficial ingredients, which may find applications in functional foods and nutraceuticals.

CRediT authorship contribution statement

Viive Sarv: Methodology, Investigation, Formal analysis, Writing – original draft; **Petras Rimantas Venskutonis**: Conceptualization, Methodology, Validation, Writing – Reviewing and editing, Supervision, Resources; **Laura Tamkutė**: Methodology, investigation, Formal analysis, Writing – original draft; **Renata Baranauskienė**: Methodology; Investigation, Formal analysis, **Dalia Urbonavičienė:** Methodology, Investigation, Formal analysis; **Pranas Viškelis**: Methodology; Formal analysis; **Rajeev Bhat**: Resources, Editing, Supervision.

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.

Acknowledgments:

This work is supported by Plantvalor Project "PlantValor–full-scale product development service in synergy with the traditional activities of Polli Horticultural Research Centre 2020–2023", which has received funding from the European Regional Development Fund; and by ERA Chair in VALORTECH project, which has received funding from the European Union's Horizon 2020 Research and Innovation Program under grant agreement No. 810630. In addition, funding was received from Mobilitas Pluss ERA-Chair support (Grant No. MOBEC006 ERA Chair for Food (By-) Products Valorisation Technologies of the Estonian University of Life Sciences). The authors also acknowledge Estonian Ministry of Rural Affairs for the programmes "Collection, Conservation and Utilization of Plant Genetic Resources in 2021–2027".

References

Acree, T., & Arnold, H. (2004, July 28). *Flavornet*. http://www.flavornet.org

Adams. (2007). *Identification of essential oil components by gas chromatography/mass spectrometry,* (4th Ed).

Adams, Cohen, S. M., Doull, J., Feron, V. J., Goodman, J. I., Marnett, L. J., Munro, I. C., Portoghese, P. S., Smith, R. L., Waddell, W. J., & Wagner, B. M. (2005). The FEMA GRAS assessment of benzyl derivatives used as flavor ingredients. In *Food and Chemical Toxicology* (Vol. 43, Issue 8, pp. 1207–1240). https://doi.org/10.1016/j.fct.2004.11.014

Adams, R. P. (2017). *Identification of essential oil components by gas chromatography/mass spectrometry, 4th ed* (R. P. Adams (Ed.); Allured Publishing). Allured Business Media.

Ahmadkelayeh, S., & Hawboldt, K. (2020). Extraction of lipids and astaxanthin from crustacean by-products: A review on supercritical CO2 extraction. In *Trends in Food Science and Technology* (Vol. 103, pp. 94–108). Elsevier Ltd. https://doi.org/10.1016/j.tifs.2020.07.016

Ali, E., Hussain, S., Hussain, N., Kakar, K. U., Shah, J. M., Zaidi, S. H. R., Jan, M., Zhang, K., Khan, M. A., & Imtiaz, M. (2022). Tocopherol as plant protector: an overview of Tocopherol biosynthesis enzymes and their role as antioxidant and signaling molecules. In *Acta Physiologiae Plantarum* (Vol. 44, Issue 2). Springer Science and Business Media Deutschland GmbH. https://doi.org/10.1007/s11738-021-03350-x

Baldino, L., Scognamiglio, M., & Reverchon, E. (2020). Supercritical fluid technologies applied to the extraction of compounds of industrial interest from Cannabis sativa L. and to their pharmaceutical formulations: A review. In *Journal of Supercritical Fluids* (Vol. 165). Elsevier B.V. https://doi.org/10.1016/j.supflu.2020.104960

Basegmez, H. I. O., Povilaitis, D., Kitrytė, V., Kraujalienė, V., Šulniūtė, V., Alasalvar, C., & Venskutonis, P. R. (2017). Biorefining of blackcurrant pomace into high value functional ingredients using supercritical CO2, pressurized liquid and enzyme assisted extractions. *Journal of Supercritical Fluids*, *124*, 10–19. https://doi.org/10.1016/j.supflu.2017.01.003

Bobinaitė, R., Kraujalis, P., Tamkutė, L., Urbonavičienė, D., Viškelis, P., & Venskutonis, P. R. (2020). Recovery of bioactive substances from rowanberry pomace by consecutive extraction with supercritical carbon dioxide and pressurized solvents. *Journal of Industrial and Engineering Chemistry*, *85*, 152–160. https://doi.org/10.1016/j.jiec.2020.01.036

Commission Regulation (EU) No 432. (2012). In *Official Journal of the European Union,*

650 *L* (Vol. 136, Issue 1). http://www.efsa.europa.eu/en/topics/topic/article13.htm

651 Doležal, M., Velíšek, J., & Famfulikova, P. (2003). Aroma of less-known wild fruits.
652 *Flavour Research at the Dawn of the Twenty-First Century-Proceedings of the 10th*
653 *Weurman Flavour Research Symposium, Beaune, France, 25-28 June, 2002. Editions*
654 *Tec & Doc*, 576–579.

655 Doležal, M., Velíšek, J., & Famfulíková, P. (2001). Chemical composition of less-
656 known wild fruits. *Biologically-Active Phytochemicals in Food: Analysis, Metabolism,*
657 *Bioavailability and Function. Proceedings of the EUROFOODCHEM XI Meeting,*
658 *Norwich, UK, 26-28 September 2001. Royal Society of Chemistry*, 241–244.

659 European Commission. (2020). *Farm to Fork Strategy 2 CONTENTS*.

660 Grunovaite, L., Pukalskiene, M., Pukalskas, A., & Venskutonis, P. R. (2016).
661 Fractionation of black chokeberry pomace into functional ingredients using high
662 pressure extraction methods and evaluation of their antioxidant capacity and
663 chemical composition. *Journal of Functional Foods*, *24*, 85–96.
664 https://doi.org/10.1016/j.jff.2016.03.018

665 Houlberg, S., Westh, B. C., & Poll, L. (2000). The aroma composition of ethanol
666 extracts of rowanberries (Sorbus aucuparia). *Frontiers of Flavour Science*, 56–57.

667 Ivakhnov, A. D., Sadkova, K. S., Sobashnikova, A. S., & Skrebets, T. E. (2019).
668 Optimization of Oil Extraction from Rowanberry Waste in Alcoholic Beverage
669 Production. *Russian Journal of Physical Chemistry B*, *13*(7), 1135–1138.
670 https://doi.org/10.1134/S1990793119070091

671 Kitrytė, V., Kavaliauskaitė, A., Tamkutė, L., Pukalskienė, M., Syrpas, M., & Rimantas
672 Venskutonis, P. (2020). Zero waste biorefining of lingonberry (Vaccinium vitis-
673 idaea L.) pomace into functional ingredients by consecutive high pressure and
674 enzyme assisted extractions with green solvents. *Food Chemistry*, *322*.
675 https://doi.org/10.1016/j.foodchem.2020.126767

676 Kitrytė, V., Narkevičiūtė, A., Tamkutė, L., Syrpas, M., Pukalskienė, M., &
677 Venskutonis, P. R. (2020). Consecutive high-pressure and enzyme assisted
678 fractionation of blackberry (Rubus fruticosus L.) pomace into functional
679 ingredients: Process optimization and product characterization. *Food Chemistry*,
680 *312*. https://doi.org/10.1016/j.foodchem.2019.126072

681 Kitrytė, V., Povilaitis, D., Kraujalienė, V., Šulniūtė, V., Pukalskas, A., & Venskutonis,
682 P. R. (2017). Fractionation of sea buckthorn pomace and seeds into valuable
683 components by using high pressure and enzyme-assisted extraction methods.
684 *LWT - Food Science and Technology*, *85*, 534–538.
685 https://doi.org/10.1016/j.lwt.2017.02.041

686 Klavins, L., & Klavins, M. (2020). Cuticular wax composition of wild and cultivated
687 northern berries. *Foods*, *9*(5). https://doi.org/10.3390/foods9050587

688 Kraujalis, P., Kraujalienė, V., Kazernavičiūtė, R., & Venskutonis, P. R. (2017).
689 Supercritical carbon dioxide and pressurized liquid extraction of valuable
690 ingredients from Viburnum opulus pomace and berries and evaluation of
691 product characteristics. *Journal of Supercritical Fluids*, *122*, 99–108.
692 https://doi.org/10.1016/j.supflu.2016.12.008

693 Kraujalis, P., & Venskutonis, P. R. (2013). Supercritical carbon dioxide extraction of
694 squalene and tocopherols from amaranth and assessment of extracts antioxidant
695 activity. *Journal of Supercritical Fluids*, *80*, 78–85.
696 https://doi.org/10.1016/j.supflu.2013.04.005

697 Kryževičiute, N., Kraujalis, P., & Venskutonis, P. R. (2016). Optimization of high
698 pressure extraction processes for the separation of raspberry pomace into
699 lipophilic and hydrophilic fractions. *Journal of Supercritical Fluids*, *108*, 61–68.
700 https://doi.org/10.1016/j.supflu.2015.10.025

701 Mu, H., & Høy, C. E. (2004). The digestion of dietary triacylglycerols. In *Progress in*
702 *Lipid Research* (Vol. 43, Issue 2, pp. 105–133). Elsevier Ltd.
703 https://doi.org/10.1016/S0163-7827(03)00050-X

704 Nattagh-Eshtivani, E., Barghchi, H., Pahlavani, N., Barati, M., Amiri, Y., Fadel, A.,
705 Khosravi, M., Talebi, S., Arzhang, P., Ziaei, R., & Ghavami, A. (2022). Biological
706 and pharmacological effects and nutritional impact of phytosterols: A
707 comprehensive review. In *Phytotherapy Research* (Vol. 36, Issue 1, pp. 299–322).
708 John Wiley and Sons Ltd. https://doi.org/10.1002/ptr.7312

709 Piironen, V., Lindsay, D. G., Miettinen, T. A., Toivo, J., & Lampi, A. M. (2000). Plant
710 sterols: Biosynthesis, biological function and their importance to human
711 nutrition. In *Journal of the Science of Food and Agriculture* (Vol. 80, Issue 7, pp.
712 939–966). https://doi.org/10.1002/(SICI)1097-0010(20000515)80:7<939::AID-
713 JSFA644>3.0.CO;2-C

714 Sarv, V., Venskutonis, P. R., & Bhat, R. (2020). The sorbus spp.—underutilised plants
715 for foods and nutraceuticals: Review on polyphenolic phytochemicals and
716 antioxidant potential. *Antioxidants*, *9*, 1–23. https://doi.org/10.3390/antiox9090813

717 Sarv, V., Venskutonis, P. R., Rätsep, R., Aluvee, A., Kazernavičiūtė, R., & Bhat, R.
718 (2021). Antioxidants Characterization of the Fruit, Juice, and Pomace of Sweet
719 Rowanberry (Sorbus aucuparia L.) Cultivated in Estonia. *Antioxidants*, *10*(11),
720 1779. https://doi.org/10.3390/antiox10111779

721 Slavin, M., & Yu, L. (2012). A single extraction and HPLC procedure for
722 simultaneous analysis of phytosterols, tocopherols and lutein in soybeans. *Food*
723 *Chemistry*, *135*(4), 2789–2795. https://doi.org/10.1016/j.foodchem.2012.06.043

724 Sukhija, P. S., & Palmquist, D. L. (1988). Rapid Method for Determination of Total
725 Fatty Acid Content and Composition of Feedstuffs and Feces. In *Food Chem* (Vol.

726 36). https://pubs.acs.org/sharingguidelines

727 Tamkutė, L., Liepuoniūtė, R., Pukalskienė, M., & Venskutonis, P. R. (2020). Recovery
728 of valuable lipophilic and polyphenolic fractions from cranberry pomace by
729 consecutive supercritical CO2 and pressurized liquid extraction. *Journal of*
730 *Supercritical Fluids*, *159*. https://doi.org/10.1016/j.supflu.2020.104755

731 Tamkutė, L., Pukalskas, A., Syrpas, M., Urbonavičienė, D., Viškelis, P., &
732 Venskutonis, P. R. (2020). Fractionation of cranberry pomace lipids by
733 supercritical carbon dioxide extraction and on-line separation of extracts at low
734 temperatures. *Journal of Supercritical Fluids*, *163*.
735 https://doi.org/10.1016/j.supflu.2020.104884

736 *The Good Scents Company Information System*. (2022, July 28).
737 http://www.thegoodscentscompany.com/

738 Venskutonis, P. R. (2020). Berries. In *Valorization of Fruit Processing By-products* (pp.
739 95–125). Elsevier. https://doi.org/10.1016/b978-0-12-817106-6.00005-8

740 Welke, J. E., Zanus, M., Lazzarotto, M., & Alcaraz Zini, C. (2014). Quantitative
741 analysis of headspace volatile compounds using comprehensive two-
742 dimensional gas chromatography and their contribution to the aroma of
743 Chardonnay wine. *Food Research International*, *59*, 85–99.
744 https://doi.org/10.1016/j.foodres.2014.02.002

745 Zeb, A., & Murkovic, M. (2010). MS / corn oil / rapeseed oil / sunflower oil. *Eur. J.*
746 *Lipid Sci. Technol.* , *112*, 844–851. https://doi.org/10.1002/ejlt.201000064

747 Zymone, K., Raudone, L., Raudonis, R., Marksa, M., Ivanauskas, L., & Janulis, V.
748 (2018). Phytochemical profiling of fruit powders of twenty Sorbus L. Cultivars.
749 *Molecules*, *23*(10), 1–17. https://doi.org/10.3390/molecules23102593

750