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Valorization of food side streams by supercritical fluid extraction of compounds of interest from apple pomace

Vénicia Numa^{a,b,*}, Christelle Crampon^a, Arnaud Bellon^b, Adil Mouahid^a, Elisabeth Badens^{a,**}

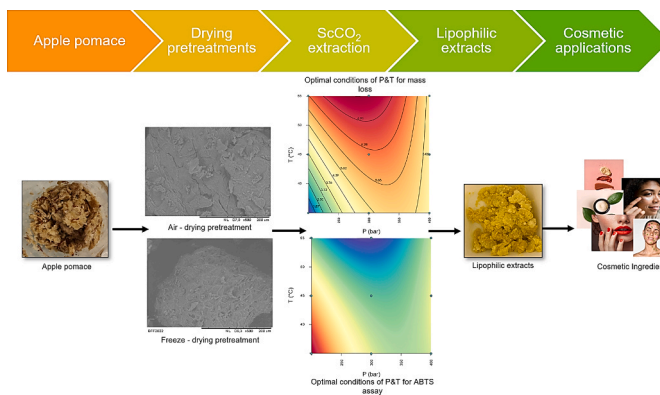
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HIGHLIGHTS

- Valuable compounds can be recovered from apple pomace using supercritical extraction.
- Best conditions for extraction of nonpolar compounds are 300 bar and 55 °C.
- Freeze-dried apple pomace exhibits an enhanced extraction kinetics.
- Fatty and terpenic acids are the most extracted compounds using pure ScCO₂.
- ScCO₂ extraction of apple pomace shows a good scalability at larger scale.

GRAPHICAL ABSTRACT



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ABSTRACT

Supercritical CO₂ (scCO₂) extraction, a green technology still little applied to side streams, has been used to explore the potential recovery of beneficial compounds from apple pomace, a food industry byproduct. The study examines the potential of scCO₂ extraction on freeze-dried and airflow dried apple pomace, using laboratory-scale equipment with varying pressures (200–400 bar), temperatures (35–55 °C) with a fixed CO₂ flow rate. Extracts were analyzed through LC-MS and GC-MS, while antioxidant capacity was assessed using the ABTS assay. The results were compared to those from Soxhlet n-hexane extraction. Optimal conditions of 300 bar and 55 °C with freeze-dried apple pomace yielded the highest mass loss. The main compounds identified included glyceryl dilinoleate, linoleic acid, and diacyl glycerol, with significant ursolic acid content. A preliminary higher scale feasibility test under optimal conditions demonstrated promising, duplicable outcomes, supporting prior claim that apple pomace contains valuable ingredients that can be reused in various industrial sectors.

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Nomenclature

e	Extraction yield at the first extraction period (kg/kg of plant).
G	Initial fraction of extract in open cells.
x_u	Concentration of extract in the plant before extraction (kg/kg of plant).
t_1	Time of the end of the first extraction period (s).
y_s	Solute apparent solubility (kg /kg solvent).
Θ_f	Dimensionless external mass transfer resistance.
q'	Specific flow rate (kg /kg plant/s).
t_i	Characteristic time of the mass transfer in the solid phase (internal diffusion) (s).
ρ_{CO_2}	CO ₂ density (kg/m ³).
U_{CO_2}	CO ₂ velocity in an empty barrel (m/s).
d_p	Mean particle diameter (m).
a_g	Specific surface area of a grain (m ⁻¹).
ε	Bed void fraction.
μ	CO ₂ dynamic viscosity (Pa.s).
τ	Tortuosity.
H/D	Ratio height/diameter of the autoclave (m/m).
TIC	Total Ion Chromatogram (%).

1. Introduction

Almost 45 % of all waste production originates from the agricultural industry. Europe is the first producer of food waste, followed by Latin America, North America and Oceania [1]. Considering the amount of waste generated each year, the valorization of side streams has become a crucial topic and a huge opportunity. However, many side streams from the food & nutrition industries can become by-products with potential interest [2]. Indeed, a by-product is a side stream produced by an industry which no longer has any interest for it, but which can be valuable in other industries. Numerous valorizations for by-products are possible in various domains such as textiles, supplementary foods, cosmetic ingredients, fertilizers, packaging, and construction materials. In this context, the valorization of agricultural by-products is of great interest due to their potential high added value in other industries.

Apple is one of the most frequently consumed fruit worldwide with about 93 million tons of production in 2021 [3] and regrettably, about 20–35% (equivalent to 4 million per year [4]) of apple leftovers can be discarded [5–7]. There are many benefits of a good management and valorization of apple wastes. The first one is to obviously limit the production of agro-food wastes which, if not managed appropriately, can be a source of environmental pollution and health risks. Furthermore, when it comes to apple pomaces, they are rich in active ingredients of interest for different applications as antioxidants, preservatives, biopesticides, anti-corrosion agents... Biopolymers, such as PHAs (poly-hydroxyalkanoates), can also be recovered from AP. In addition of all these valuable ingredients, AP are now considered as feedstocks for diverse bioenergy applications, targeting the production of biofuels, biogas, pyrolysis oil, biochar or activated carbon [4]. The value chain of AP can be then mapped with two main distinct blocks: i) separation treatments for the recovery of active compounds that can be considered as medium to high added value ingredients, ii) transformations for producing biomass fuels and bioenergy materials. If a focus is made on the former block, the recovery of apple bioactive compounds exhibiting potential antimicrobial, antibacterial, anti-inflammatory, anti-analgesic, antimycotic, antioxidant, and tonic properties [2,5,8,9] is of great interest. Apple pomace, which is made up of the peel, flesh, stems, calyx, and core of the fruit, contains a variety of bioactive compounds including dietary fibers, carbohydrates, sugars, as well as phenolic and lipidic compounds [5,6,10,11–13]. These

compounds are largely valued for their properties as gelling agents or antioxidants [14]. Inside the cuticular waxes of apple, there are mainly non-polar compounds, potentially recoverable as by-products [15]. Ursolic acid and erythrodiol, which are major components of apple epicuticular wax, have anti-inflammatory, antioxidant, and tyrosinase inhibition properties [2,8]. These numerous properties can be interesting especially in pharmaceutical or cosmetic industries and can also be valuable in helping to reduce the use of petroleum wax [16]. However, it has been shown that the content and ratio of lipid compounds in apple pomace may change during storage [17]. For example, the content of palmitic and oleic acid may increase, while the content of linoleic acid decreases in Golden Delicious apples during storage. As Golden Delicious apples generally have the highest content of fatty acids among other varieties, this variation in lipid content can impact the extraction results. These different compounds of interest can be extracted using non-conventional techniques of extraction such as supercritical CO₂ (scCO₂) [2,17–19].

The use of scCO₂ extraction could be helpful in reducing the environmental print of this holistic valorization. Indeed, the use of organic solvents for extraction techniques is increasingly being banned, particularly to meet the regulations set forth by the REACH Regulation [20]. ScCO₂ extraction is a clean and renewable technique, due to the numerous properties of CO₂ (non-toxic, non-flammable, abundant and economic) [21]. With this technique, the separation of the solvent from the extract occurs spontaneously during depressurization [14]. Its selectivity for lipophilic compounds is another advantage in the context of extracting compounds from apple pomace, including triterpenic acids (lupeol, friedelanone, erythrodiol, ursolic acid), alkanes (pentacosan, nonacosan), sesquiterpenes, fatty acids (linoleic acid, linolenic acid, oleic acid), and phytosterols (β -sitosterol) [19]. All these lipophilic compounds present in apple waxes can have diverse applications including the use of fatty acids in soaps, detergents, and polishes, as well as the use of resin acids such as antifungal agents in cosmetics and paints [16]. Also, their protective role against environmental factors is largely known [8]. Working with a by-product whose composition, physical aspect, and behavior are highly variable, during extraction is a major challenge. Only a few studies have been conducted on the use of scCO₂ for extracting non-polar compounds from apple pomace. Table 1 provides an overview of the most relevant papers that have dealt with scCO₂ extraction from apple peel, apple seeds, and pomace [18,19,22,23]. The different drying pretreatments performed on apple biomass were airflow drying (55 or 60 °C), freeze-drying (at –45 °C) or oven drying (40 °C). To the best of our knowledge, only one study has been conducted specifically on apple pomace from Golden Delicious variety [22]. This latter work has highlighted that the optimal operating conditions of scCO₂ extraction are 300 bar and 46 °C, with a corresponding extraction yield of 3.27 % and with extracts rich in triterpenic acids and fatty acids. In addition to the recovery of lipidic compounds, some publications highlighted the presence of phenolic compounds in apple pomace (phloridzin, quercetin, chlorogenic acid, and others) which can be extractable with scCO₂ and co-solvents [8,22,20,24]. This technique coupled with the optimal co-solvent could be very efficient in comprehensively valorizing all apple compounds [25].

The present article is focused on apple pomace valorization using scCO₂ technology and this study has been led by an industrialist involved in the production of apple pomace and able to reuse it internally in its cosmetic branch. The aim of this work is more specifically to investigate the potential of scCO₂ extraction for recovering valuable products from apple pomace, while establishing and optimizing the conditions for the drying pretreatment and for the extraction. Freeze-drying and airflow drying are the most commonly used drying techniques for food products and thus, both were studied in this work [27]. Freeze-dried (FD) and airflow dried (AD) apple pomaces were compared in terms of extraction yields and compositions of extracts obtained using either scCO₂ or Soxhlet extraction with n-hexane. An experimental design focused on scCO₂ extraction was then carried out over a range of

Table 1Experimental operating conditions of scCO₂ extraction studies performed on apple pomace from the literature.

Reference	Biomass type	Pretreatments	Conditions of extraction	Optimal conditions	Compounds identified	Results
Wozniak et al., 2018[18]	Apple pomace (<i>Malus Domestica Borkh</i>)	Airflow dried at 55 °C	T:40–80 °C; P:100–300 bar; flow rate: 4.17.10 ⁻⁴ – 12.5.10 ⁻⁴ L/s;	P: 300 bar, T: 80 °C	Triterpenic acids, Phytosterols	Yield of triterpenic acids 0.95 %
De la Peña Armada et al., 2021[22]	Apple pomace (Golden Delicious)	Freeze-dried at – 45 °C	15 g of pomace; P: 300, 425, 550 bar; T: 37, 46, 55 °C; 100 min; flow rate: 0.6 kg/h	P: 300 bar; T: 46 °C	Triterpenic acids, Fatty acids	Extraction Yield: 3.27 %
Li et al., 2015[19]	Apple peel (Red Fuji)	Airflow dried at 60 °C; d _p : 200 µm	100 g of peels; T: 50 °C; P: 350 bar; 90 min	P: 350 bar; T: 50 °C	Fatty alcohols, Alkanes, Fatty acids, Esters, Triterpenoids, Aldehydes	Extraction Yield: 2.08 %
Montañes et al., 2018[26]	Apple seeds (Jazz & Braeburn)	No pretreatments; d _p : 0.5–1 mm	165 g of seeds; P: 300 – 1300 bar; T: 42.85–62.85 °C; 300 min, flow rate: 6–10 mL/min	P > 750 bar; T < 52.85 °C	Fatty acids, Tocopherols	Extraction Yield: 21–22 % of oil
Ferrentino et al., 2020[23]	Apple seeds (<i>Malus Domestica Borkh</i>)	Oven dried at 40 °C	80 g of seeds; P: 100 – 300 bar; T: 40–60 °C; flow rate: 1–8 L/h	P:240 bar; T:40 °C	Phenolic compounds, Fatty acids	Yield: 20.5 µg/g _{oil} DPPH: 0.71 mg Trolox/g _{oil}

pressure (200 – 400 bar) and temperature (35 – 55 °C) at a fixed scCO₂ flow rate (0.14 kg/h) to study the influence of these variables on the extraction yield and the extract composition. In addition, in order to better understand the behavior of the biomass under scCO₂ and to evaluate mass transfer limitations, extraction kinetics were plotted for some selected conditions. The extraction curves were modeled using the simplified Broken and Intact Cells (BIC) model developed by Sovová [28]. Analytical techniques such as LC-MS and GC-MS were used to analyze the extracts obtained after *n*-hexane and scCO₂ extractions. The antioxidant capacity of the extracts was also measured using the ABTS assay. Ursolic acid, as a compound of interest and rather concentrated in apple pomace, was quantified in the different extracts. Finally, scCO₂ extraction was performed at a larger scale (autoclave volume *100) under the optimal conditions identified at lab scale. The data collected will aid a further scale-up of the process for industrial uses. An energetic and economic analysis ends the discussion to evaluate the potential of ScCO₂ extraction for valorizing compounds of interest from apple side-streams.

2. Materials and methods

2.1. Raw material and chemicals

Apple pomace (var Golden Delicious, *Malus Domestica Borkh*) was

processed in September 2020 from Diana Food (Symrise Nutrition) in France. The pomace, containing mixed seeds, peels, stem, and more than 50 % of water, was received frozen in batches of 1 kg. ScCO₂ extraction requires to be applied on dry matter and two drying methods largely used in food industry were chosen for comparison: freeze-drying and airflow drying. For the freeze-drying method, a laboratory scale equipment (RP2V, SGD Serail Argenteuil, France) was used at – 55 °C and 30 ± 10 Pa for approximately 40 h. For the airflow drying method, a laboratory scale air-dryer (Homeday) was used at 45 °C for approximately 24 h. The dry pomace was then ground with a mechanical grinder and sieved to obtain batches of different granulometries. The most abundant mean particle diameter after sieving (d_p) was 400 µm (Fig. 1) and this mean granulometry was used for the different extractions. Samples were stored at – 19 °C in amber glass bottles until further use. The water content of the ground biomass was estimated to be under 5 wt% for both freeze-dried and airflow dried samples, through the measurement of the mass loss for a 2 g sample after oven drying (Mettler, Shawbach, Germany) at 105 °C for 48 h. Measurements were taken at the same time to calculate the water content on wet basis. CO₂ was provided by Air Liquide (France), with a purity of 99.7 %. *n*-hexane was provided by VWR, HPLC grade (France) and used to perform Soxhlet extraction. A cleaning step of the pipes was carried out after scCO₂ extraction with dimethylsulfoxide solvent (DMSO) and then ethanol (96 %).

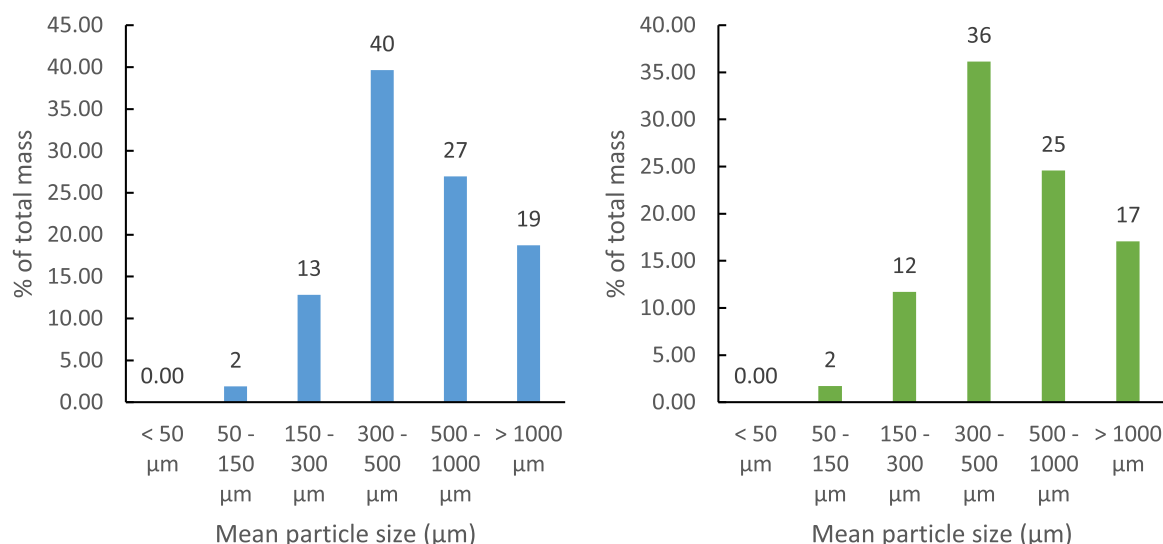


Fig. 1. Mean particle size for freeze-dried (blue) and airflow dried (green) apple pomace.

2.2. Extractions

2.2.1. Soxhlet extraction

Soxhlet extractions were conducted with *n*-hexane (99.8 %, Carlo Erba, France) to compare the composition of hexane extracts with that of scCO₂. About 150 mL of solvent were used for 10 g of freeze-dried apple pomace. The Soxhlet apparatus was set up and heated to the boiling temperature of the solvent for 8 h. After extraction, the extract was separated from the solvent under a rotary evaporator (Heidolph Laborota 4000) at temperature between 40 and 50 °C under vacuum, weighed, and stored at -19 °C until analysis. The yield was calculated with Eq. (1). The experiments were repeated three times to confirm the results. The residual biomass was dried in the oven to remove the solvent.

$$\text{Extraction Yield}(\%) = \frac{\text{mass of extracted oil}(\text{kg})}{\text{mass of biomass introduced}(\text{kg})} \times 100 \quad (1)$$

2.2.2. ScCO₂ Extraction: experimental setup

The experimental setup (Fig. 2) is a laboratory scale equipment from Separex (Champigneulle, France) aiming to work in semi-continuous conditions. The extraction autoclave, a stainless-steel vessel of 20 mL (internal length: 16 cm, internal diameter: 1.2 cm), is filled with a sample mass between 4 and 6 g, closed and heated up to the desired working temperature. CO₂ (1) in liquid phase (purity of 99.7 % from Air Liquid, France) previously cooled in a cryogenic bath (2) at -4 ± 2 °C, is filtered and pumped (3) at the desired pressure (4), through a thermostated enclosure (5) into the extraction vessel (6). This extraction vessel has two flat stainless-steel frits that allow the circulation of the CO₂-rich phase containing the extract. A time of approximately 10–30 min is necessary to reach the desired experimental conditions of temperature and pressure. The flowrate, controlled with the pump and the expansion valve, is kept constant during the extraction, and measured with a flow meter placed at the end of the extraction line. Two following separators are also included at the end of the extraction line, after the expansion valve. In the first separator (7), a glass tube is placed to recover the extract. In the second one (8), a tube with glass beads filled with ethanol and placed in a cold trap helps to catch the volatile compounds.

The extraction yield has been calculated from the mass loss of the extractor (Eq. 2). Indeed, since these experiments have been performed on a small-scale apparatus, if the extraction yield is calculated considering the mass of extract recovered in the separators, a significant error

can be made if some compounds are trapped in the tubing placed between the extractor and the separators. For such conditions, the extraction yield estimation is more accurate by double weighing the extractor. The measurement of the mass of the biomass in the autoclave before and after the extraction was performed with a weighing accuracy of 10⁻² g.

$$\text{Mass loss} = \frac{\text{initial mass}(\text{kg}) - \text{mass after extraction}(\text{kg})}{\text{initial mass of biomass}(\text{kg})} \times 100 \quad (2)$$

The scCO₂ flow rate chosen (0.14 kg/h) has been determined from previous works; it corresponds to a contact time between the biomass and the solvent sufficient enough to allow good mass transfers [29].

In order to characterize the CO₂ flow regime in the extractor, the modified Reynolds number was calculated. The aim was to work in similar flow conditions at the different scales studied here (autoclaves of 20 mL and 2 L). The modified Reynolds number (Re'_p) used to characterize the flow during scCO₂ extraction in porous medium is defined as following:

$$\text{Re}'_p = \frac{\rho_{\text{CO}_2} U_{\text{CO}_2}}{(1 - \epsilon) a_g \mu_{\text{CO}_2}} \quad (3)$$

$$\text{With } a_g = \frac{6}{dp} \quad (4)$$

The CO₂ density was determined from the NIST website [30] and the calculated value of bed void fraction for the studied apple pomace was 0.8 (experimental determination using the method of Mouahid et al., [31]) for a mean particle size of 400 μm. With a modified Re < 1, the CO₂ flow is considered as linear laminar, between 1 < Re < 10, the flow regime is considered as intermediate and above 10 as non-linear laminar. A linear laminar or intermediate flow is recommended for a stable and more predictable extraction with scCO₂, avoiding CO₂ channeling or axial dispersion. Indeed, a linear laminar flow corresponds to a CO₂ plug flow which is expected for an optimal contact between CO₂ and the biomass.

2.3. Modeling

2.3.1. Modified BIC model

Different mathematical models, more or less complex, are reported in the literature, based on empirical equations or mass balance into the extraction bed [29,30,32]. Sovová's equation in terms of characteristic

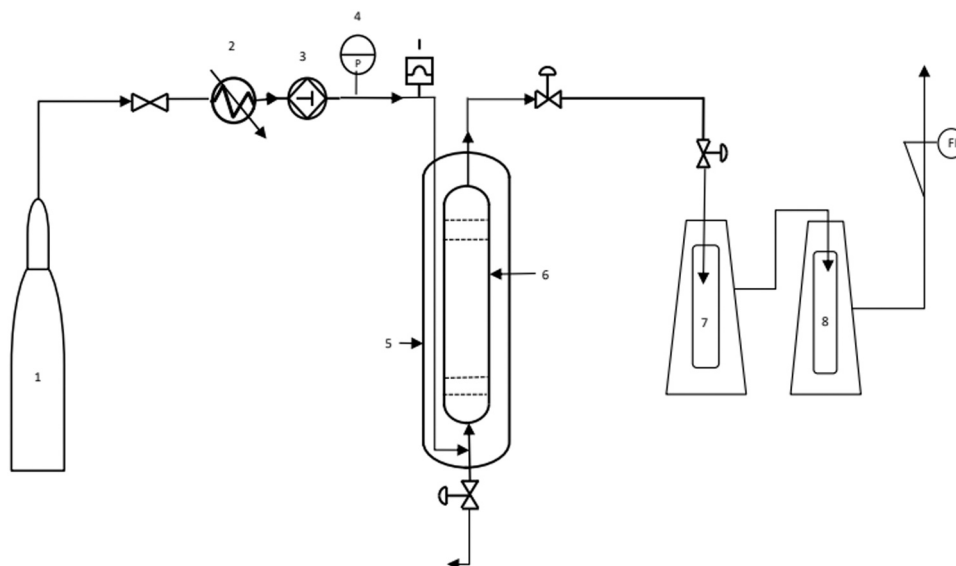


Fig. 2. Process diagram of scCO₂ extraction setup.

times was used in this study to analyze the results obtained [33]. A simplification of the equations was made to plot the extraction curves based on the use of characteristic times for all the physical processes for botanical materials [28]. Type A curve of Sovová's BIC model was used. The first part of the extraction curve corresponds to the extraction of the accessible oil in the open cells, meaning that the extraction yield (e) is equal to the product of the initial fraction of oil in the open cells with the extract concentration before extraction: $e = G \cdot x_u$ at $t = t_1$, the first period of extraction, with:

$$t_1 = \frac{G \cdot x_u}{y_s \{1 - \exp(-1/\theta f)\} q} \quad (5)$$

The second part of extraction depends on the internal diffusion with the corresponding equations:

$$e = x_u \left[1 - (1 - G) \exp\left(-\frac{t - t_1}{t_i}\right) \right] \quad \text{for } t > t_1 \quad (6)$$

The parameter θf is adjusted from the linear part of the extraction curve and t_i is fitted from the second part of the extraction curve using respectively Eq. (5) and Eq. (6). The last squares method was used to determine the adjustable parameters. The other parameters G , t_1 and e are estimated regarding the shapes of the extraction curve [28].

The modeling was performed using Matlab. The curves are presented in terms of mass loss (kg/kg) as a function of the CO_2 /biomass mass ratio (kg CO_2 /kg biomass).

2.3.2. Experimental design

The influence of operating conditions (pressure and temperature) upon the extraction efficiency was investigated using a complete factorial experimental design and data were compiled using Azurad software. The responses investigated to evaluate the extraction efficiency were the mass loss obtained after extraction, the antioxidant capacity (IC50%), and the content in ursolic acid (%) (Table 2). Among the studied responses, the mass loss was prioritized. Therefore, the best extraction conditions were considered as those leading to the highest mass loss (%). The experiments were performed randomly to limit the possible variabilities. A second-order polynomial model was used for each response (Eq. 7).

$$Y = B_0 + B_1 X_i + B_2 X_j + B_{11} X_i^2 + B_{22} X_j^2 + B_{12} X_i \cdot X_j \quad (7)$$

Where X_i and X_j are pressure and temperature, respectively, and the coefficient of the polynomial model B_0 (central coefficient), B_1 and B_2 (coefficient quantifying the effects of pressure and temperature), B_{1-1} , B_{2-2} and B_{1-2} (the coefficients quantifying the interactions). Three levels were considered in this experimental plan corresponding to the pressure (200, 300 and 400 bar), the temperature (35, 45 and 55 °C) at the same flow rate 0.14 kg/h on apple pomace FD. All extraction durations were set at 90 min.

2.4. Scale-up parameters for scCO_2 at lab scale 2

Another scale (lab scale 2) supercritical CO_2 extraction was conducted using 400 g of freeze-dried apple pomace that was sieved under

Table 2
Experimental design.

Experiment	Pressure (bar)	Temperature (°C)	CO_2 density (kg/m ³)
1	200	35	865.72
2	200	55	754.61
3	400	35	972.26
4	400	55	906.77
5	300	35	929.11
6	300	55	850.22
7	200	45	812.69
8	400	45	939.75
9	300	45	890.33

similar conditions as for lab scale 1 extraction. The scale-up corresponds to a size 100 times larger for the autoclave volume. The biomass was placed in a 2 L autoclave (25.4 cm for height and 8.1 cm for the inner diameter), which was pressurized using a CO_2 pump and then heated through a hot heat exchanger. The CO_2 was recycled in a loop throughout the batch. After extraction, the autoclave was depressurized to recover the residual biomass and measure the mass loss. The extract was recovered during extraction inside the separators, by isolating the extraction vessel from the rest of the circuit. The parameters for the lab scales 1 and 2 and extractions are presented in Table 3. Analyzing these parameters will allow the identification of the key-criteria to be considered for a successful extrapolation. The duration at both scales were chosen to have a similar CO_2 /biomass ratio at the end of the extractions (2 h corresponding to a CO_2 /biomass ratio of 50). The mass loss of extraction was measured after double weighing of the autoclave after extraction (weigh accuracy of 10^{-2} g).

There are various approaches to study the scale-up of an extraction process [30,31]. One option is to maintain the same solvent-to-biomass ratio at which the extraction reaches its maximum efficiency, best compromise between the mass loss and the duration (or solvent consumption), and then calculate the solvent flow rate based on the initial biomass mass. Another option is to keep the same residence time in the autoclave, and a third option is to maintain the same velocity of solvent within the autoclave. In this study, the same ratio CO_2 /biomass was kept at the two different scales. Moreover, similar CO_2 velocities were used, and, in both scales, the flow patterns corresponded to a linear laminar regime.

2.5. Analytical methods

2.5.1. Antioxidant capacity

The antioxidant capacity was measured with ABTS Assay, a cell free in vitro test. The principle is based on the reductive decolorization of the green colored cationic radical 2,2'-Azinobis-3-ethylbenzothiazoline 6-sulfonic acid (ABTS^{•+}) by antioxidants. The decolorization is then measured at 734 nm with a spectrophotometer. For the protocol, 20 μl of each test sample, the organic solvent, and a control solution were mixed in a 96-well microtiterplate in duplicates. Then, the addition of potassium persulfated led to the oxidization of ABTS^{•+}, which was further left for incubation in the dark at 30 °C. The results are expressed in % of the IC50 value, meaning that the lower the value, the higher the extract antioxidant capacity. The details of the protocol used are the property of the company Symrise.

2.5.2. LC-MS analysis

Liquid Chromatography is performed by a Waters Acquity Ultra High Performance (LC) device and a Bruker micrOTOF II device coupled with a mass spectrometry (MS) detector (ThermoFisher Scientific) (positive or negative ionization and UV) and a Charged Aerosol Detector (CAD),

Table 3
Scale up parameters of scCO_2 extraction used for FD apple pomace at 300 bar and 55 °C.

	Lab scale 1	Lab scale 2
H/D	13.2	2.7
Biomass mass (kg)	$4.5 \cdot 10^{-3}$	0.40
Autoclave volume (L)	0.02	2
Apparent biomass density (kg/m ³)	227.00	
CO_2 density(kg/m ³)	850.22	
Bed Porosity	0.80	
Filling ratio (%)	99	88
Solvent (CO_2) Velocity (m/s)	$6.3 \cdot 10^{-3}$	$5.7 \cdot 10^{-3}$
Modified Reynolds number (Re'_p)	1.3	1.6
Solvent (CO_2) flow rate(kg/h)	0.14	10.00
Extraction time (min)	95	120
CO_2 /Biomass mass ratio (kg/kg)	50	
Mean particle size (μm)	400	

generating a mass spectrum for the identification of the different compounds. This latter is performed with a Kinetex RP-C18 column, 100 mm × 2.1 mm, and a thickness of 1.7 μm. Based on the literature, the relative standard deviation (RSD) for the detection of these compounds is 3 % [34]. The results are expressed in wt%. When needed, the extracts were centrifuged before measurement to facilitate the analysis. This method was used to have relative values based on integration and not the exact quantified values. A similar method is used for the quantification of Ursolic acid (UA), using a reference of UA for the determination of the concentration. The details of the protocol used are the property of the company Symrise.

2.5.3. GC-MS analysis

Gas chromatograph (GC) coupled to mass spectrometer (MS) is a broad applied technique used in analytical areas. Before measurement the extracts were cleaned up by centrifugation. The separation of the sample was done by the GC (e.g., Nexus GC 2030, Co. Shimadzu) with a capillary column (e.g., ZB-1 ms, 60 m × 0.18 mm ID × 0.18 μm film thickness, Co. Phenomenex). The ionization, detection and signal processing done by the MS (e.g., QP2020 NX, Co. Shimadzu). The analysis data were reported by creating a labeled chromatogram and a result table with qualitative composition of the detected substances with their percentage of all analytes (TIC in %). The targeted compounds are the non-polar, volatiles and semi-volatiles compounds inside the extracts. The details of the protocol used are the property of the company Symrise.

2.5.4. Scanning electron microscopy (SEM)

Observations of the biomass, after scCO₂ extraction experiments, were made using scanning electron microscopy (Hitachi TM3000 Tabletop microscope). A metallization (thin layer of Au-Pd during 30 s) was performed on the samples dropped on an adhesive pastille.

3. Results and discussion

3.1. Soxhlet extraction

The total extract yield from apple pomace using *n*-hexane extraction was 0.18 kg oil/kg biomass for freeze-dried samples, after 8 h of extraction (Table 4). It is not meaningful to compare these results to those from other studies due to the variability in the origin and processing mode of the biomass used [22]. The yield from the *n*-hexane extraction was higher than that from scCO₂ extraction, which is likely due to the lower selectivity of *n*-hexane and the longer extraction time (8 h vs. 90 min for scCO₂ extraction). In this study, *n*-hexane extraction using the Soxhlet method was used as a reference technique for comparison with scCO₂ extraction in terms of composition. The antioxidant capacity obtained for the Soxhlet extracts was similar to the one obtained for the optimal conditions of scCO₂ extraction (0.015 % and 0.056%IC₅₀, respectively), indicating that both methods are effective for the extraction of antioxidant compounds. Since high content of ursolic acid was observed in the Soxhlet extract, a good extraction of triterpenic acids can be expected with scCO₂.

3.2. Supercritical fluid extraction

3.2.1. Effect of pretreatments upon scCO₂ extraction performance from apple pomace

The supercritical CO₂ extraction curves in Fig. 3 demonstrate the impact of pretreatment on the extraction kinetics and mass loss. The results are presented in terms of mass loss (kg/kg) as a function of the CO₂/biomass mass ratio (kg CO₂/kg biomass) for airflow dried (AD) and freeze-dried (FD) apple pomace under the same operating conditions (mean particle size of 400 μm, pressure of 300 bar, temperature of 55 °C, and CO₂ flow rate of 0.14 kg/h). The choice of this flow rate for all the experiments was determined after previous works performed

Table 4

Results obtained using an experimental design, using as responses: the mass loss of extraction (wt%), the antioxidant capacity with ABTS (% IC₅₀) and the quantification of ursolic acid (%), for scCO₂ extraction performed on FD apple pomace for 90 min. The results are also presented for the Soxhlet with *n*-hexane on FD apple pomace for 8 h.

Experiment	Pressure	Temperature	Mass loss	ABTS	Ursolic
	(bar) X ₁	(°C) X ₂	(wt%) Y ₁	(IC ₅₀ %) Y ₂	acid (%) Y ₃
1	200	35	2.99 ± 0.050*	> 0.1	4.08 ± 0.037*
2	200	55	5.75 ± 0.026	> 0.1	2.82 ± 0.053
3	400	35	5.1 ± 0.029	> 0.1	2.45 ± 0.061
4	400	55	4.50 ± 0.033	> 0.1	2.47 ± 0.061
5	300	35	3.31 ± 0.045	0.072 ± 0.15	2.68 ± 0.056
6	300	55	8.64 ± 0.017	0.056 ± 0.15	3.37 ± 0.045
7	200	45	5.78 ± 0.026	> 0.1	2.09 ± 0.072
8	400	45	4.66 ± 0.032	> 0.1	2.11 ± 0.071
9	300	45	6.56 ± 0.023	> 0.1	3.04 ± 0.049
Control	Soxhlet hexane FD	69 ± 10	18 ± 0.25	0.015	15.3

* Coefficient of variation (%)

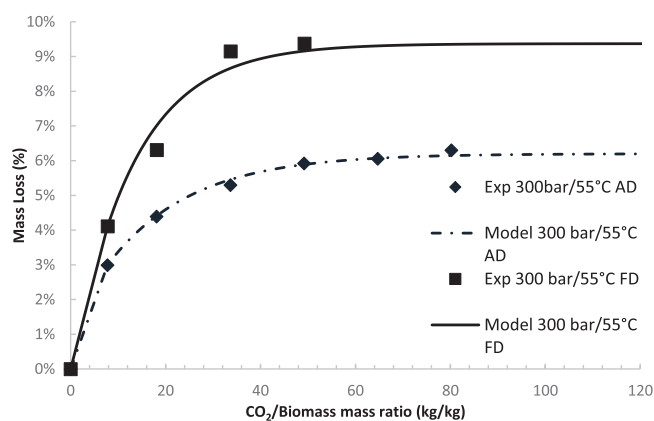


Fig. 3. Experimental apple pomace extraction curves for freeze and airflow dried (45 °C) pretreatments, at 300 bar and 55 °C.

with the extraction apparatus at lab scale (Separex) [26,30,35]. At a CO₂/biomass ratio of 50, the AD apple pomace led to a maximal mass loss of around 6.0 %, while the FD pomace led to mass loss of around 9.0 %. The air-drying pretreatment was therefore less effective than the freeze-drying technique. The FD sample provides the highest mass transfer and the highest mass loss compared to the AD apple pomace.

These preliminary results regarding FD pretreatment are confirmed with the study made by Ferrentino et al. [9]. The drying method used can significantly affect the composition, stability, and shelf life of apple pomace over time. Drying at 45 °C using the airflow drying technique can result in degradation of the product and its compounds in the long term.

In addition, observations of SEM images of apple pomace after scCO₂ extraction were made to compare the effect of the drying technique on the biomass (Fig. 4). The residues after airflow drying (a) and freeze drying (b) showed different surface appearances, with the presence of cavities in the freeze-dried pomace and a smoother surface for the airflow dried pomace. These observations confirm the suggestion made, that freeze drying pretreatment has a positive impact on the extraction

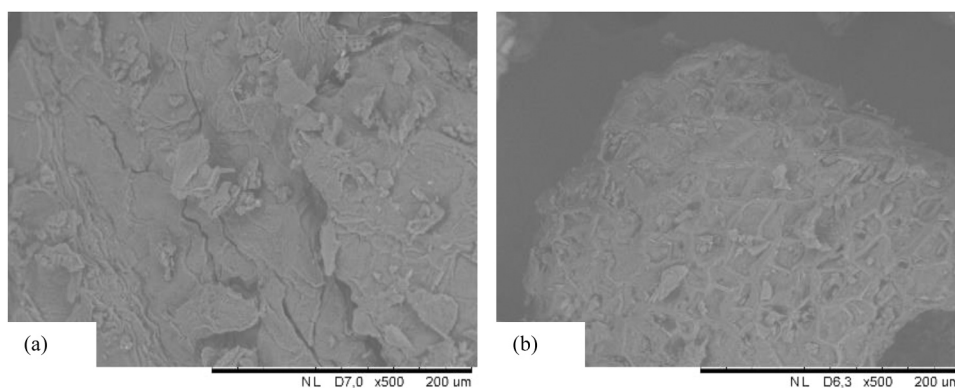


Fig. 4. SEM images of apple pomace after extraction (300 bar, 55 °C) for AD (a) and FD pomace (b).

mass loss due to increased accessibility of the oil in the biomass. All the results observed on apple pomace, extraction curves and SEM pictures, showed the positive impact of FD as an efficient technique for the pre-treatment of apple pomace. The experimental plan was therefore made on FD samples, to measure the mass loss of extract, the antioxidant capacity and ursolic acid content. On another note, the energetic impact of freeze-drying must be taken into account for the development of larger scale extractions.

3.2.2. Repeatability tests

Different sets of repeatability tests were performed on FD apple pomace at different conditions: 300 bar and 55 °C, 400 bar and 60 °C, with a flow rate of 0.14 kg/h (Fig. 5). The conditions 400 bar and 60 °C were selected due to the close CO₂ density with one of the optimal conditions (Table 4) - 850 kg/m³ at 300 bar and 55 °C and 890 kg/m³ at 400 bar and 60 °C. The tests performed showed a good repeatability of the extraction curves 5.4 % for 300 bar and 55 °C and 7.6 % for 400 bar and 60 °C).

3.2.3. Effects of pressure and temperature upon scCO₂ extraction of apple pomace

The results in Table 4 highlight the influence of pressure and temperature on the extraction mass loss and the quality of the corresponding extract (antioxidant capacity and ursolic acid content) after 90 min of extraction using freeze dried apple pomace. These results were compared to those obtained using Soxhlet extraction with *n*-hexane on freeze dried biomass. In this work, the extraction efficiency was finally defined using mass loss (%) as a criterion. Therefore, the best extraction

conditions were considered as those leading to the highest mass loss (%).

The results concerning the composition of the extracts at the best operating conditions using LC and GC-MS are presented in Table 6 and Table 7, respectively.

The mass losses for the extracts obtained using scCO₂ extraction vary significantly over the entire range of pressure (200–400 bar) and temperature (35–55 °C) studied, as well as the antioxidant capacity and the content of ursolic acid (UA). The highest mass loss obtained for scCO₂ extraction was 8.64 %, at 300 bar and 55 °C. Similar moisture content has been measured before and after extraction on the residue, confirming that the mass loss obtained is not partly due to water extraction. There were no significant variations in the composition of the extracts in the range studied, but previous research has shown that pressure can significantly affect the extraction of neutral lipids, such as triterpenic acids and fatty acids, due to an increase in CO₂ density when pressure increases [36]. Similar optimal conditions for extracting triterpenic acids from apple pomace using supercritical CO₂ were found to be 300 bar and 46 °C in the study of De la Peña Armada et al., which used a range of 300–550 bar, 37–55 °C, 15 g of apple pomace, and a flow rate of 10 g/min for 100 min. These operating conditions yielded a mass of 3.27 [22].

In other studies, it was observed that increasing pressure resulted in lower selectivity and higher extraction yields [37]. This could explain the high mass losses obtained in this study. However, it is important to note that the scales and extraction duration of these studies were not the same, and the apple pomace used did not come from the same source, so the comparisons are only partially relevant. In the study by De la Peña Armada et al., the authors used an experimental design that resulted in a

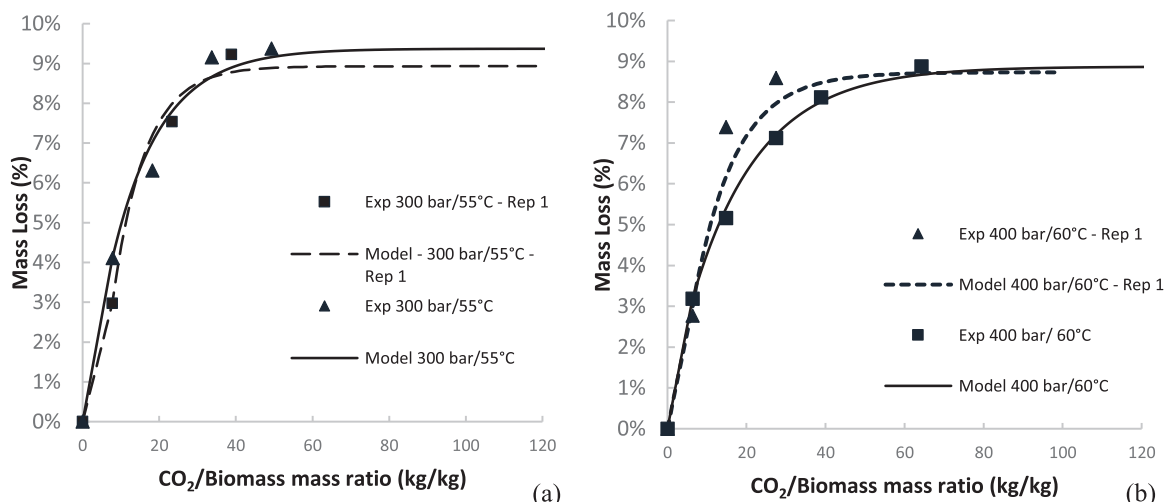


Fig. 5. Repeatability curves performed and modeled on FD apple pomace at 300 bar/55 °C (a) and 400 bar/60 °C (b).

polynomial model, allowing them to find optimal conditions where the responses reached their maximum values at specific temperatures and pressures [22]. For our study, the results showed that scCO₂ extraction can be effective in recovering valuable products from apple pomace at moderate pressure and temperature. This optimal region may be due to the low solubility of the compounds at lower pressure and the decrease in mass transfer efficiency and consequently, the extraction mass loss at higher pressure. Some extracts had high antioxidant capacity, while the value of IC50 didn't go above 0.1 %. The highest antioxidant capacity was observed at 300 bar and 55 °C, which may be related to the highest mass loss achieved during extraction. In a previous study, De la Peña Armada et al. found that the optimal conditions for Oxygen Radical Absorbance Capacity (ORAC) and Hydroxyl Radical Antioxidant Capacity (HORAC) were 425 bar and 46 °C and 37 °C, respectively [22].

To support these findings with statistical results, Table 5 gathers the coefficient values of the second-order polynomial model (Eq. 7) for the influence of pressure and temperature on the mass loss. The corresponding regression coefficient for mass loss response is 0.68.

The p-values obtained for the different parameters indicate that both pressure and temperature are significant for mass loss response, with a confidence of 5% for the effect of temperature and 1 % for the pressure. The interactions between both coefficients have also a confidence of 1 % (temperature-temperature and pressure-temperature). Besides, the values obtained for the other answers (antioxidant capacity and ursolic acid content) didn't exhibit significant p-values, with regression coefficients of 0.62 and 0.19, respectively.

The results showed in Fig. 6 confirmed the optimal conditions observed, by the highest mass loss and the best antioxidant capacity. The surface response for antioxidant capacity also matched the results for the optimal extraction parameters, with the lowest IC50 observed at 300 bar and 55 °C. When considering only the content of ursolic acid, the optimal conditions were found to be 200 bar and 35 °C (4.08 % ± 0.037). However, the content of ursolic acid at 300 bar remains satisfying with 3.37 % ± 0.045.

The extraction curves obtained at these optimal conditions were compared to those obtained at 400 bar and 60 °C to analyze the behaviors of apple pomace extraction under other conditions of pressure and temperature but corresponding to close CO₂ densities. The extraction curves are presented in Fig. 7.

Since the results are similar, the lowest conditions of pressure and temperature were highlighted as the optimal conditions regarding environmental and energy consumption aspects. The modeled parameters of the curves are presented and discussed in Section 3.2.5.

3.2.4. Characterization of apple pomace by LC-MS and GC-MS

The results of the analysis for scCO₂ extract at the optimal conditions and the Soxhlet *n*-hexane extract showed that the main compounds identified using LC-MS (Table 6) for the scCO₂ extract were fatty acids, with more than 33% of average quantity (diacyl glycerol, linoleic acid, oleic acid, linolenic acid, palmitic acid, stearic acid, and ursolic acid). Among these compounds, linoleic acid was the most abundant with 12.28 %. This composition and average content of fatty acid for this variety was expected in literature [38]. In contrast, the *n*-hexane extract

Table 5

Coefficients of the second-order polynomial model and analysis of the variance for the effects of pressure and temperature on the mass loss.

Coefficients	Coefficients values	Standard deviation	p value %
B0	6.15	0.33	-
B1 (pressure)	1.30	0.17	* **
B2 (temperature)	0.48	0.19	*
B1-1	0.02	0.34	95.93
B2-2	-1.95	0.31	* **
B1-2	-1.19	0.22	* **

*p < 0.005;

* **p < 0.001

identifies more triterpenic acids than fatty acids, such as oxopomolic acid, pomolic acid, pomonic acid, or erythrodiol. Ursolic acid was the most abundant compound detected in this extract, with 53 wt%. The scCO₂ extract also contained unknown compounds and relatively low levels of ursolic, ficulinic, stearic, linolenic, arachidic, and palmitic acids. Ursolic acid has potent biological activities, including anti-proliferative and antiangiogenic properties as well as other triterpenic compounds [39]. All the retention times (RT) are localized between 15 and 25 min for the scCO₂ apple extracts and 12–25 min for the soxhlet extracts.

A GC-MS analysis was conducted to identify others compounds in the extracts, where LC-MS solvents were not suitable for their solubilization. The results are shown in Table 7, with the compounds organized by family.

The CO₂ extract obtained at 300 bar and 55 °C using scCO₂ contains different alkanes, such as nonacosan, octacosan, pentacosan, hentriacontan, or heptacosan, as well as fatty alcohols (C24-C29), aldehydes, fatty aldehydes (hexacosanal, octacosanal, tetracosanal), fatty esters, terpenoids (lupeol, friedelan-3-one), fatty acids (palmitic acid, linoleic acid, oleic acid, stearic acid), phytosterols (β-sitosterol), and esters. Some compounds were only identified with their molecular mass, retention time, and are grouped in the "unknown" category. Lupeol and friedelan-3-one also known as friedelin, detected as triterpenic acids, present significant protective activities (inhibition of the production of reactive oxygen species, hepatoprotective activity) [39]. Fatty aldehydes, fatty alcohols and fatty acids, are important intermediaries in the production of waxes [40]. Regarding the identified phytosterols, a study showed the beneficial effects of β-sitosterol as a good anti-inflammatory agent, as well as functional properties for cosmetic products (interfacial activity, emulsifying properties) [19]. The *n*-hexane extract contained similar compounds, including alkanes, terpenoids, and phytosterols, but no fatty acids or alcohols. The presence of more triterpenic acids was expected in the CO₂ extract given the general composition of apple pomace, but differences in methodology, storage time, biomass origin, and extraction process may explain the observed differences in composition [2,18,22].

3.2.5. Modeling of extraction curves: BIC parameters

The simplified model proposed by Sovová was applied to the data presented in Fig. 3, Fig. 5 and Fig. 7 [31]. The same methodology was followed as in the work of Mouahid et al. [36]. The BIC parameters for the different modeled curves are shown in Table 8.

In Fig. 3, the extraction curves were modeled to better understand the effects of pretreatment on scCO₂ extraction. Also, as the values obtained for y_s are significantly different at 300 bar and 55 °C for FD biomass, (5.28 vs 3.83), it is not meaningful to compare these values with the apparent solubility calculated with the AD biomass in the same operating conditions.

Regarding the impact of the extraction operating conditions (Fig. 7), it can be considered that BIC parameters are similar, for both conditions. Indeed, considering the variations occurring with the repeatability curves made in the same conditions, the results can be globally smoothed and considered as similar, since apple pomace is a side stream that can exhibit variable characteristics.

3.2.6. Scale-up study

The extraction results obtained at a larger scale (autoclave volume × 100) using the optimal conditions determined at lab scale (300 bar and 55 °C) and with a flow rate of 10 kg/h, showed an extraction yield of 8.2 %. This is rather close to the results obtained at lab scale 1, which were an extraction yield of 9.4 % (as shown in Fig. 8 and the corresponding curve at the optimal conditions in Fig. 7). The deviation between both results is 12 % which is relatively satisfying. The flow characteristics, as measured by the Re_p parameter, were similar in both lab scale 1 and 2 extractions, with values of 1.3 and 1.6, respectively, indicating a linear laminar regime. The same ratio of solvent to biomass of 50 was chosen

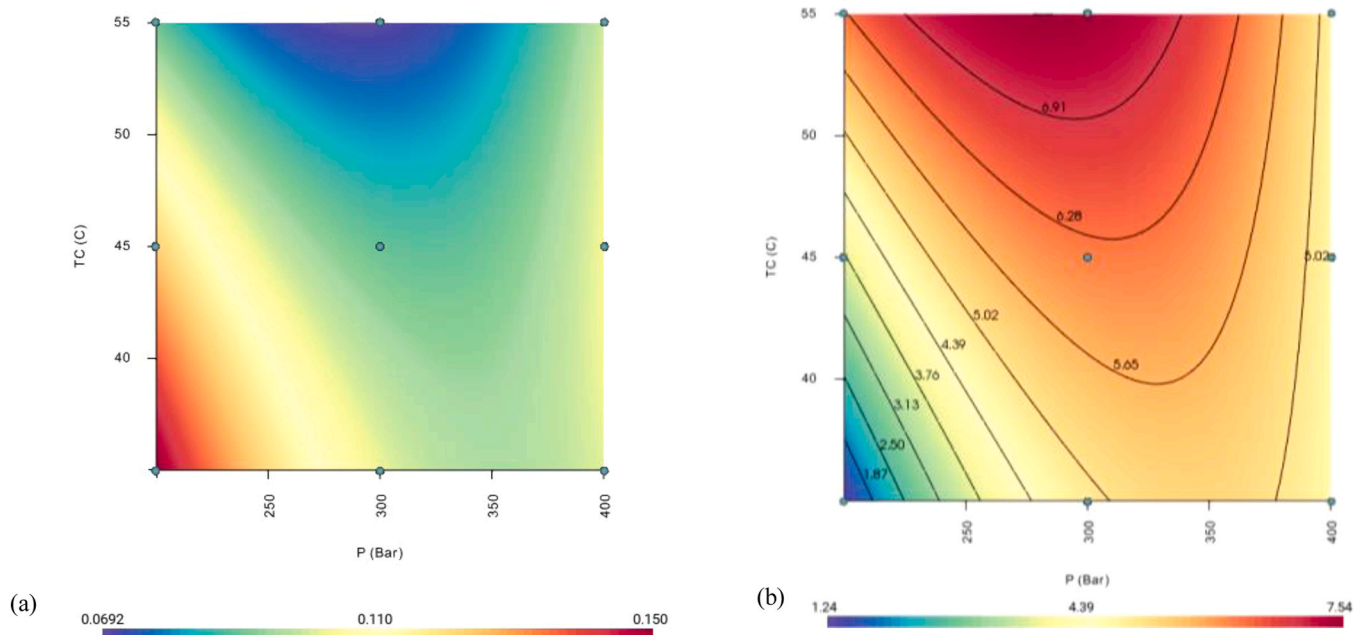


Fig. 6. Two-dimensional surface responses of the antioxidant capacity (%IC50) (a) and the mass loss of extraction (%) (b) as a function of pressure and temperature for a CO₂ flow rate of 0.14 kg/h for apple pomace.

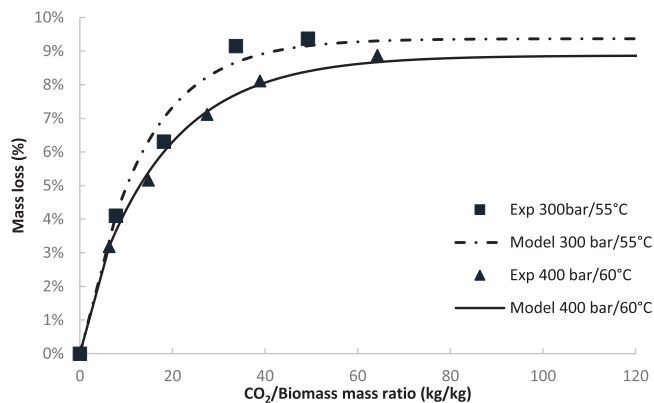


Fig. 7. Experimental extraction curves obtained for FD apple pomace at 400 bar and 60 °C and 300 bar and 55 °C.

Table 6

Relative composition of scCO₂ extracts (300 bar and 55 °C for lab scales 1 and 2 and compared to Soxhlet *n*-hexane extract) from apple pomace based on uHPLC-CAD Integration after identification through LC-MS.

Compounds	Soxhlet <i>n</i> -hexane (wt%)	CO ₂ extract at lab scale 1 (wt%)	CO ₂ extract at lab scale 2 (wt%)
Triterpenic acids (Oxopomolic acid,...)	70	2	9
Fatty acids (Linoleic acid, ...)	-	20	50
Aldehydes (Betulinic aldehyde, ...)	5	-	-
Esters (Glyceryl dilinoleate,...)	9	12	-
Unknown	16	56	41
Total	100		

for the two extractions. This value of 50 is typically in the range of the solvent to biomass ratio used at the industrial scale for supercritical extraction or fractionation, this range being 10–100 [41]. The slight

Table 7

Family of compounds identified with GC-MS technique (*n*-hexane and CO₂ extract at lab scales 1 and 2 at 300 bar and 55 °C) with their average relative TIC %, for FD apple pomace extracted with ScCO₂ at the optimal parameters.

	Soxhlet <i>n</i> -hexane (TIC%)	CO ₂ extract at lab scale 1 (TIC%)	CO ₂ extract at lab scale 2 (TIC%)
Alkanes (Pentacosan, ...)	76	33	49
Alcohols (Hexacosanol, ...)	-	17	18
Aldehydes (Tetracosanal, ...)	1	14	15
Esters (Farnesyl ester, ...)	1	4	-
Triterpenic acids (Lupeol, ...)	4	2	1
Fatty acids (Linoleic acid, ...)	-	2	-
Phytosterols (β-sitosterol)	4	2	1
Unknown	14	26	16
Total	100		

Table 8

BIC parameters.

		G	e ₁ (kg oil/kg biomass)	Θ _f	t _i (s)	t ₁ (s)	X _u (kg/kg of plant)	Y _s (g/kg CO ₂)
300 bar - 55 °C FD	Exp A	0.44	0.04	0.02	1495	900	0.09	5.28
	Exp A'	0.33	0.03	0.02	980	900	0.09	3.83
400 bar - 60 °C FD	Exp B	0.36	0.03	0.02	2447	900	0.09	5.02
	Exp B'	0.34	0.30	0.02	1487	900	0.09	4.70
300 bar - 55 °C AD	Exp C	0.48	0.03	0.02	2060	900	0.06	3.86

A' - B': Repeatability extraction curves (mean curve presented)

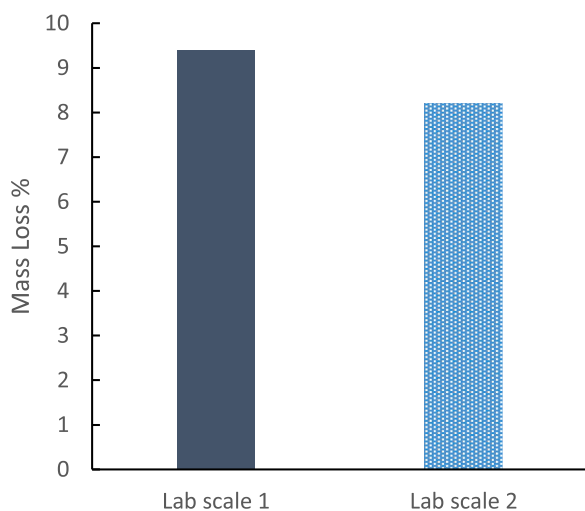


Fig. 8. Mass loss (%) obtained for lab scale 1 and 2, for FD apple pomace at 300 bar and 55 °C.

differences in the results between the two scales can be attributed to the differences in the apparatus used, such as the ratio of autoclave height to diameter (respectively 13.2 and 2.7), leading to performance differences. A potential loss of extract within the tubing at the larger scale can be also assumed. Other studies have shown good scalability for apple pomace extraction using supercritical CO₂, with optimal parameters of 300 bar and 80 °C, and a flow rate of 8.69.10⁻³ L/s, when using a 500 mL autoclave at larger scale [18].

The composition of the extract obtained at larger scale was analyzed using LC-MS and GC-MS (Table 6 and Table 7). The results show a similar composition to that obtained at lab scale, although there were some variations for certain compounds. This could be attributed to the higher quantity of extract that was available for analysis at larger scale, allowing for better identification of compounds. The GC-MS analysis revealed that around 50 % of the major compounds identified were alkanes, followed by fatty alcohols, fatty aldehydes, triterpenic acids, and phytosterols. This is consistent with the results obtained in this study at lab scale. Moreover, other studies have also highlighted a similar composition than our study at lab scale [16,19].

All the results reported in the literature and obtained in this study showed promising results for the scalability of this process to recover compounds of interest from apple pomace.

4. Energetic and economic analysis of the process including pretreatment and extraction of valuable compounds

From an energy perspective, a research was conducted by Ludwig et al. [42] to compare the energy and cost elements for recovering β-carotene from *Dunaliella salina* biomass using n-hexane and scCO₂ + ethanol as cosolvent (at 300 bar, 50 °C and during 180 min) extraction methods [42]. The study performed at pilot scale, highlighted that scCO₂ extraction required more extraction energy (57.42 kWh/kg of β-carotene) than n-hexane extraction (28.88 kWh/kg of β-carotene). However, when looking at the overall energy production (considering solvent consumption, evaporation of the solvent from extract, ...), both methods were equivalent (562 kWh/kg for scCO₂ and 542.57 kWh/kg for hexane). In terms of total costs, both methods were also equivalent, with scCO₂ extraction costing slightly less (147.98\$/kg) than hexane (153.08\$/kg) [42]. In this work, no cosolvent was used with scCO₂, a diminution of both energy consumption and production costs can be then expected.

Regarding the energy consumption of studied drying pretreatments, FD consumes more energy than AD (approximately 1.8 kWh per kilogram of water removed against 1 kWh per kilogram of water,

respectively) [43]. FD proved more appropriate for various reasons, including the preservation of heat-sensitive compounds, widespread use in the food industry, and greater amount of compounds extracted considering the results of this work. Freeze-drying is already a standard process for high-value products in the food industry. Hence, its use in this context is justified only if the extracts are of certain added value.

The different compounds present in apple pomace can be highly valuable for different applications as mentioned in introduction. Concerning the nonpolar compounds and waxes extracted, the main applications are exploited in cosmetic products, such as the alcohols for their sebum reduction properties, anti-dandruff or anti-bacterial properties [44]. Industrial applications for the functional properties of apple waxes as haircare and conditioning agents are also valued by companies [45]. These different applications to value apple by-products could be highly beneficial for a switch from a linear to a circular economy.

5. Conclusion

This study examined the potential of using scCO₂ extraction to recover valuable compounds from apple pomace, focusing on the impact of pretreatment, of the extraction conditions as well as the process scale on the extraction yield and antioxidant capacity of the extracts. The optimal extraction conditions were found to be 300 bar and 55 °C, resulting in a mass loss of 8.64 % and an IC50 of 0.056 %. The main compounds identified in the extracts were fatty acids, along with triterpenic compounds such as ursolic acid, lupeol, friedelin and esters. The highest content of ursolic acid in the extract was obtained under different optimal conditions (200 bar and 35 °C). Freeze-drying was found to be the most effective pretreatment method in terms of extraction yield and kinetics but had no significant impact on antioxidant capacity. Extraction curves were successfully fitted by the BIC modified model of Sovová with a certain variability due to the source of the biomass. This study has clearly demonstrated the potential for using scCO₂ extraction to valorize apple pomace, having achieved its initial goals. Despite the inherent instability and variability of apple pomace as a by-product, the results showed that pretreatments and scCO₂ extraction are effective in extracting valuable compounds. The presence of a diverse range of compounds further highlights the richness of apple pomace as a potential source of valuable compounds. The results obtained at a larger scale were consistent with the lab-scale ones, suggesting a possibly good scalability of the process. By-products of these compounds hold great potential for high added value in the cosmetic industry, making it an attractive sector for targeting their utilization. For future works, experiments with green co-solvents such as ethanol or water, could be done for the extraction of polar fractions such as antioxidants, sugars, polysaccharides, fibers, or carbohydrates from apple pomace. The residues from the successive extractions can be finally transformed, used thus as feedstocks for various bioenergy applications. Preliminary energetic and economic analysis indicates that scCO₂ extraction may be viable to valorize compounds of interest from apple side-streams.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Venicia Numa reports financial support was provided by Symrise AG. Venicia Numa reports a relationship with Symrise AG that includes: employment.

Data availability

Data will be made available on request.

Acknowledgments

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