Bruno Aguilar Veiga, Fabiane Hamerski, Mathias Porsmose Clausen, Massimiliano Errico, Agnes de Paula Scheer, Marcos Lúcio Corazza, Compressed fluids extraction methods, yields, antioxidant activities, total phenolics and flavonoids content for Brazilian Mantiqueira hops, The Journal of Supercritical Fluids, Volume 170, 2021, 105155.

Compressed fluids extraction methods, yields, antioxidant activities, total phenolics and flavonoids content for Brazilian Mantiqueira hops

Bruno Aguilar Veiga[a], Fabiane Hamerski[a], Mathias Porsmose Clausen[b], Massimiliano Errico[b], Agnes de Paula Scheer[a], Marcos Lúcio Corazza[a,*]

[a] *Department of Chemical Engineering, Federal University of Paraná, Francisco H. Santos Av. 100, 81531-990, Curitiba, PR, Brazil.*

[b] *Department of Green Technology, University of Southern Denmark, Campusvej 55, 5230 Odense M, Denmark*

Abstract

This study compares the yields of different extractions methods for a Brazilian variety of *Humulus lupulus* using $scCO_2$, $scCO_2$+ethanol, $scCO_2$+ethyl acetate, and compressed propane. Extracts were characterized by total phenolics content (TPC), total flavonoid content (TFC), and antioxidant activity (AA). The extraction yields were from 2.7 to 10.1 wt% when using compressed propane at 20 °C and 10 MPa and $scCO_2$+ethyl acetate at 80 °C and 25 MPa, respectively. Furthermore, the effect of adding ethyl acetate as a cosolvent in a semi-batch process over the extraction time and extracts properties were evaluated in comparison to the extracts obtained using $scCO_2$, $scCO_2$+ethanol, and compressed propane. The results indicate that ethyl acetate increases the extraction yield and produces samples with higher TPC, TFC, and AA values comparing to $scCO_2$+ethanol and similar values compared to pure $scCO_2$.

Keywords: *Humulus lupulus*; antioxidant activity; supercritical CO_2; compressed propane; cosolvent.

1. Introduction

Humulus lupulus is a dioic, perennial, herbaceous plant from the *Cannabaceae* family, native to Europe, West Asia, and North America. It is classified as a vine and can reach 6.1 m in height [1]. Only female plants produce the inflorescence known as hops, where the lupulin glands are found. Lupulin is rich in essential oils and resins. Essential oils are responsible for the aroma of the beer, and resin content provides bitterness. The bitter compounds are mainly alpha and beta acids, the most abundant components in the resin, and these acids become water-soluble after an isomerization reaction providing bitterness to the beers [2,3].

Applications of hops as a medicinal plant date back to the 19th century [4]. Researchers have confirmed hops as an anti-inflammatory [5] and phytoestrogen source [6–8]. *Humulus lupulus* flowers are used to relieve the symptoms of stress and insomnia [4] and as antifungal agents [9]. In vitro studies have shown that hops have anticarcinogenic and anti-fibrogenic activity in liver tissue [10,11]. The essential oil obtained from hops is a mixture of more than 35 compounds, including organic acids, alcohols, terpenes, esters, and phenolics [4].

Phenolic compounds are byproducts of plant metabolism, acting as natural protection against environmental conditions. These compounds are classified as primary antioxidants due to their role as reducing agents, hydrogen donors, and singlet oxygen suppressants [12,13]. Hops contain phenolic compounds that are classified as phenolic carboxylic acids (ferulic acid), flavonoids, and polyphenols. Among the polyphenols, resveratrol and xanthohumol are the most abundant prenylflavonoids [4].

Methods mostly used for obtaining oil-containing extracts from vegetable raw materials are the techniques involving solid-liquid extraction with organic solvents and steam distillation. Methods using organic solvents demand a separation step to recover the solvent, and during the solvent distillation, a significant part of the volatile components can be removed from the final extract. The second mentioned method demands high temperatures, which can compromise the quality of the extract due to the degradation of the thermolabile compounds [14]. On the other side, supercritical fluid extraction (SFE) and pressurized fluid extraction (PFE) are alternative techniques due to lower temperature employed, high extraction rates. In the first case, the separation of the solvent from the extract is facilitated due to the high volatility of the fluid at low-pressure conditions producing a solvent-free extract [15,16]. Compressed propane has also been proposed as a viable nonpolar solvent for lipids extraction from natural matters. Several studies with propane as a solvent in PFE have shown good extraction yields, and high antioxidant activity for the final extracts obtained [17–25].

The main limitation of SFE and PFE using pure supercritical carbon dioxide (scCO_2) and propane concerns its low solvation capacity for polar compounds. However, the addition of polar modifiers (polar cosolvents) can overcome this limitation and thus contribute to increasing the overall extraction yield [26,27]. In this sense, He et al. [28] showed that the use of ethanol as a cosolvent favored the extraction of flavonoids from hop residues leading to an increase in flavonoid recovery from 0.5 to 7.6 mg/g at the optimal concentration of cosolvent, 80 % of ethanol to sample mass ratio. However, other modifiers should be tested to optimize the extraction of phenolics. Magalhães et al. [29] tested five different solvents, and the methanol presented the highest recovery (92%) for xanthohumol, the most abundant prenylflavonoid present in the hops, followed by ethyl acetate with a recovery of 69%. Based on these previous studies, ethyl acetate as a cosolvent to the carbon dioxide for hop extraction seems to be a promising technique, mainly because it is a GRAS solvent with a low boiling point, and it is capable of providing high extraction yields.

Another aspect worth mentioning is that repeated studies in the literature have used hop varieties cultivated in such traditional regions as the United States, England, Czech Republic, and Belgium [29–32]. This study focuses on a new variety of hops developed and adapted to the climatic conditions in Brazil. This subspecies could generate extracts with distinct characteristics concerning other traditional hops from temperate regions. To the best of our knowledge, there are no reports in the literature combining the use of this new variety of hops locally produced with developing and promoting green technologies for the industry. Therefore, this work aimed to evaluate the extraction of hops using supercritical CO_2 ($scCO_2$), supercritical CO_2 + ethanol ($scCO_2$+EtOH), supercritical CO_2 + ethyl acetate ($scCO_2$+EtOAc), and compressed propane at different temperatures and pressures, and compare the extracts obtained from the extraction with compressed solvents to those obtained by Soxhlet extraction with different organic solvents. The extraction results were evaluated in terms of extraction yield, antioxidant capacity (AA), total phenolic compounds (TPC), and total flavonoid compounds (TFC).

2. Material and Methods

2.1. Samples

The vegetable matrix selected for this study was the flowers of *Humulus lupulus* Brazilian varietal, Brazilian hops (BH), which were purchased from a small hops grower cooperative in Brazil. This varietal is a mutation adapted to the Brazilian climate and originated from an American hop known as Cascade, which in turn is the result of the selective crossing with the Fuggle hop varietal from England. The samples were collected in Tuiuti (state of São Paulo – Brazil), located at an elevation of 790 m above the sea level, with a monthly average precipitation of 140 mm and temperatures varying from 11 to 26 °C. The coordinates of the production field are latitude S 22°49’22.6” and longitude W 46°40’10.1”. The hops were freeze-dried in a L101 Liobras (São Carlos, SP, Brazil) equipment for 30 hours under temperature and pressure conditions of -51 °C and 13.33 kPa, resulting in raw material with moisture and volatile compounds of 8.6 ± 0.5 g/100 g of freeze-dried hops. The samples named freeze-dried Brazilian hops (FBH) were stored in polyethylene bags in a freezer at -18 °C until use.

2.2. Chemicals

Ethanol 95% purity, ethyl acetate 99.5% purity, methanol 99.9% purity, and n-hexane 99.5% purity, all purchased from Neon (Suzano, SP, Brazil) used for extraction. For the supercritical and subcritical extractions, CO_2 99.5% purity and propane 99.5% purity were purchased from White Martins (Araucaria, PR, Brazil). For the total phenolics content, total flavonoids content and antioxidant activity analysis: DPPH - (2,2-diphenyl-1-picrylhydrazyl), Trolox - (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) standard, gallic acid standard, catechin hydrate standard, and Folin-Ciocalteu phenol reagent 2 N all purchased from Sigma-Aldrich (St. Louis, MO, USA), Sodium Carbonate Merck, (Darmstadt, HE, Germany) and Methanol HPLC grade Panreac (Barcelona, CT, Spain).

2.3. Determination of moisture, volatile compounds, and density

The moisture and volatile compounds were determined by weight loss upon drying, where the samples were oven-dried at 105 °C until they reached the constant weight, and the moisture was calculated by dividing the mass loss by the initial mass of the sample.

The real density of the dried raw material was determined by an automatic helium pycnometer Ultrapyc 1200e Quantachrome (Boynton Beach, FL, USA) at the Analytical Centre of the Institute of Chemistry at Unicamp, Campinas, Brazil.

2.4. Soxhlet extractions

Soxhlet extraction with different solvents was used as a benchmark extraction for reference and comparison purposes. All extractions were performed in triplicate at the boiling temperature of the solvent at atmospheric pressure for 8 h using 5 g of FBH and 180 mL of solvent. The solvents used individually for each extraction were: ethanol, ethyl acetate, methanol, and n-hexane. The samples were rotary evaporated (RV 10 Digital IKA, Staufen, BW, Germany) at vacuum conditions. After that, the extracts were dried using an air circulation oven at 60 °C until they reached a constant weight. Equation 1 was used to calculate the extraction yields.

$$Yield(\%) = \frac{Mass\ of\ extract}{Mass\ of\ matrix} \cdot 100\% \qquad (1)$$

2.5. Extraction with compressed fluids

In this work, the compressed fluid extraction methods were divided into three groups to evaluate the influence of different approaches when applied to hops: (i) extractions using supercritical CO_2 ($scCO_2$), (ii) compressed propane, and (iii) using CO_2 plus liquid solvents, consisting of $scCO_2$ with ethanol and ethyl acetate liquid solvents. All these extraction experiments were performed in a semi-batch approach.

The extraction equipment utilized in this study has been presented and described in previous studies [33–35]. The laboratory-scale extractor setup consists of a high-pressure jacketed-vessel, a 62.4 cm^3 stainless steel extraction vessel, with 19 mm diameter and 220 mm bed height, coupled to a heat circulation bath, and a syringe pump (500D ISCO, Lincoln, NE, USA) coupled to a circulation bath set to 10 °C for all extractions performed in this study. The solvent flow, during the dynamic extraction, was controlled by modulating two valves, a needle valve and a micrometer valve couple to the exit extraction vessel stream. The samples were collected in a separation flask, where the compressed fluid is separated from the sample mixture at atmospheric pressure and temperature due to the high vapor pressure of the compressed solvent. Sensors and transducers were used to measure the temperature and pressure inside the extractor.

To evaluate the influence of two main variables on the overall extraction yield, temperature and pressure, a 2^2 factorial design with triplicate determinations at the central point was carried for each system. The variables studied ranged from 40 to 80 °C and 15 to 25 MPa for $scCO_2$ and $scCO_2$+ethanol (or ethyl acetate), 20 to 60 °C and 3 to 10 MPa for compressed propane extractions. The extraction yield from each experimental condition was calculated according to equation 1.

2.5.1. Supercritical fluid extraction (SFE) and pressurized fluid extraction (PFE)

Around 10 g of FBH was packed inside the extractor vessel, and the temperature was set to extraction temperature. The pressurized fluid was pumped to the extractor until the system reached the extraction pressure set point. The matrix and the solvent were kept in contact for 60 min without flow, i.e., in a static extraction period to thermal and mechanical equilibration.

The solvent flow was then adjusted until a continuous flow of 2.0 ± 0.4 cm^3/min of solvent, measured at the syringe pump conditions. At this point, the dynamic extraction step started. In this setup, the solute-solvent mixture is depressurized through a heated micrometer valve until it reaches the atmospheric temperature and pressure in the separation flask. The mass of extract was collected in test tubes at predetermined times and used to calculate the overall extraction yield curves, according to the procedure reported by Correa et al. [36]. The total extraction time depends on each extraction procedure, and they will be listed along with extraction conditions in the discussion of the results.

2.5.2. Supercritical CO_2 + cosolvent extractions

In this study, ethanol and ethyl acetate were used as a cosolvent with $scCO_2$, acting as polarity modifiers. Ethyl acetate was used at a cosolvent to FBH mass ratio (MR) of (1:1) and (2:1). The organic solvent (ethyl acetate or ethanol) was added to the raw material inside the extractor vessel before the compressed solvent was pumped. The best three extraction conditions using $scCO_2$ + EtOAc were compared to extractions using $scCO_2$ + EtOH, as the ethanol is the most frequent cosolvent used in SFE applied to the food industry, including hop extractions [32,37].

For these extraction procedures, approximately 10 g of FBH was packed inside the extractor bed at the extraction temperature. Then the cosolvent was added to the solid inside the vessel to reach the predetermined MR. Thus, pressurized CO_2 was injected using the syringe pump until the extractor reached the extraction pressure. This condition was kept for 30 min to thermal and mechanical equilibration and to ensure the liquid phase becomes homogeneous. As previously mentioned, this step is named static extraction period. After the static extraction, the dynamic extraction took place, and the extracts were collected following the same procedure described in section 2.5.1.

An additional step for the organic solvent separation from the extract was performed in these experiments. The cosolvent (ethanol or ethyl acetate) was evaporated from the extract in an air circulating oven at 40 ± 2 ºC for 30 h.

2.6. Total phenolics content, total flavonoids content, and antioxidant activity

2.6.1. Preparation of samples

All samples were prepared and analyzed in triplicate. Extracts from hops using pressurized fluids extraction methods were weighed, approximately 50 mg of each sample, and then 10 mL of methanol was added before the analysis. This mixture was vigorously shaken for 5 min and centrifuged for 10 min at 1010 g and 25 ºC. The methanol phase was used to determine the total phenolic content, total flavonoid content, and antioxidant activity by spectrophotometric methods described below. All analyses were conducted using a UV-VIS spectrophotometer GTA 97 Global Analyzer (Monte Alto, SP, Brazil).

2.6.2. Total phenolic content by Folin-Ciocalteu reagent

Total phenolic content (TPC) was determined by the Folin-Ciocalteu reagent method and was performed according to the procedure described by Singleton et al. [38] with some modifications. Firstly, the methanol sample solution, prepared as previously described, was diluted to a final concentration of 0.7 mg mL^{-1}. To determine the TPC, 0.2 mL of the final sample solution and methanol to complete the volume up to 0.5 mL was mixed with 2.5 mL of Folin-Ciocalteu reagent (diluted 1:10 in distilled water). The mixture was kept in the darkness for 3 min. Afterward, 2 mL of sodium carbonate 7.5 % was added, and the mixture was incubated in the dark for two hours. Then, the solution absorbance was measured at 760 nm. The quantitative results were calculated using an analytical curve of gallic acid and were expressed as mg of Gallic acid equivalents (GAE) per 1 g of sample (mg GAE.g^{-1}).

2.6.3. Total flavonoid content

The total flavonoid content (TFC) of samples was determined based on the method proposed by Zhishen et al. [39], with some modifications. The aliquots ranging from 0.1 to 0.3 mL of samples were top-up with methanol reaches 0.4 mL of volume, then 1.6 mL of distilled water and 0.12 mL of $NaNO_2$ (5% w/v) were added to amber bottles and mixed. After 5 min, 0.12 mL of $AlCl_3$ (10% w/v) was added; and after 6 min, 0.8 mL of NaOH (1 $molL^{-1}$) and 0.96 mL of distilled water were added. The solution absorbance was measured at 510 nm after 5 min. Catechin was used as the standard for a calibration curve, and the results were expressed as mg of catechin equivalent (CE) per 1 g of sample (mg CE.g^{-1}).

2.6.4. Radical scavenging activity by DPPH$^\bullet$ assay

The antioxidant activity (AA) was determined by the DPPH$^\bullet$ assay performed based on the method described by Brand-Williams et al. [40]. A 3.9 mL aliquot of a 6×10^{-5} mol L^{-1} DPPH$^\bullet$ methanolic solution was mixed with 100 μL of diluted samples. The DPPH$^\bullet$ absorbance was monitored at 515 nm after one hour. The quantification was performed using a Trolox analytical curve, and the results were expressed as mmol of Trolox equivalents antioxidant capacity (TEAC) per 1 g of sample (mmol TEAC.g^{-1}).

2.7. Statistical analysis

Results were analyzed using analysis of variance (ANOVA) at a confidence level of 95 % using the software Statistica 10 (Statsoft Inc., USA), and to plot the response surfaces and evaluate the statistical model significance fits for each solvent.

3. Results and Discussion

3.1. Vegetable matrix and extractions

Vegetable matrix (FBH) used in all extraction experiments performed in this study presented a moisture content (after freeze-drying) and real density of 8.6 ± 0.5 wt% and 0.96 ± 0.01 g/cm^3, respectively. The extraction yields in Soxhlet extraction with organic solvents are presented in Table 1. The results indicate an increase in yield by increasing the polarity of the solvent. The highest extraction yield was observed for ethanol and methanol, around 25.8 wt% for both solvents, followed by the ethyl acetate (around 11.4 wt%) and n-hexane (around 6.7 wt%). Results presented in Table 1 show that this vegetable matrix presents a higher amount of extractable compounds in polar solvents, using short-chain alcohols and a short-chain ester (ethyl acetate), which is a food-grade compound. Therefore, based on results presented in Table 1, it can be seen that the ethyl acetate (EtOAc) is a promising volatile short-chain ester to be used as cosolvent and polarity modifier for hop extractions driven by supercritical CO_2 conditions, even presenting an overall extraction yield almost half of the values found for short-chain alcohols.

Another critical aspect of the results presented in Table 1 is the extraction with n-hexane, a nonpolar solvent. Besides, this solvent promoted the lowest extraction yield, compared to the other three polar solvents, and provided a valuable recovery of extracts from the hops raw material investigated. In this sense, the extractions with compressed propane, a compressed nonpolar solvent, were performed, and its extracts were compared to the extractions with $scCO_2$. These results were presented and discussed later.

Table 1 – Extraction yields of FBH extraction by different organic solvents.

Experiments	**Solvent**	**Polarity Index** [a]	**Yield (wt%)** [b]
S1	Ethanol	5.2	25.8 ± 0.9
S2	n-Hexane	0.0	6.7 ± 0.2
S3	Ethyl acetate	4.3	11.4 ± 0.6
S4	Methanol	6.6	25.8 ± 0.7

[a] Ref. [41]. [b] Extraction yield expressed in wt% (mean ± standard deviation) based on triplicate experiments.

To evaluate the influence of using different solvents and compare the results with a polarity modifier-assisted extraction, extractions with pure $scCO_2$ (SFE), $scCO_2$ + EtOH, and $scCO_2$ + EtOAc and compressed propane (PFE) were also performed. As mentioned, the fixed bed for all extractions contained approximately 10 g of FBH. Table 2 presents the overall extraction yields, calculated at the end of the semi-batch extraction process (after static extraction, plus dynamic extraction and bed depressurization steps). For SFE, PFE, $scCO_2$ + EtOAc extractions with MR of (1:1) and (2:1), the same factorial design with two factors and two levels and a triplicate at the central point was utilized. The SFE showed overall extraction yields ranging from 4.7 to 7.6 wt%. Kupski et al. [17] reported similar yields between 1.2 and 7.1 %, even using a different variety of hops, Hallertau Mittelfrüh, in pellet form.

Lower overall extraction yields were observed for the extraction using $scCO_2$ and compressed propane compared with other methods. Comparing the best yield condition of $scCO_2$ and compressed propane, the extraction yields were around 7.6 and 6.0 wt%, respectively, and it can be seen that the extraction yield obtained with $scCO_2$

was 27 % higher than the value obtained with propane. Extractions with compressed propane reached similar Soxhlet extraction values with n-hexane, indicating that the compressed propane could be used as an alternative and selective solvent for the extraction of nonpolar compounds.

Table 2 also presents the results for the extractions using $scCO_2$ + polar solvents. It can be seen that both cosolvents ethyl acetate and ethanol, presented a positive effect over the overall extraction yield. EtOAc and EtOH in (2:1) MR improved the extraction yield by 34 and 39 %, respectively, compared to the extraction results obtained using $scCO_2$. The pressurized extraction techniques provided extraction efficiency of over 80 % for at least one set of pressure and temperature of each experimental design tested (solvent type and approach used) comparing to the Soxhlet extraction (see Table 2).

Each extraction method was evaluated independently by analysis of variance (ANOVA). Figure 1 depicts the Pareto chart for all extraction results of different methods presented in Table 2. First, SFE using just $scCO_2$ as the solvent, where the most relevant factor was the pressure, with a positive effect, i.e., the increase in the pressure of extraction leads to an increase in the overall extraction yield. The temperature only presented a positive effect when combined with the pressure.

For PFE extractions with compressed propane, a positive effect of temperature and a negative effect of pressure on the yield was observed (Figure 1b), and the combination of the two independent variables resulted in an increase in the extraction yield. For the extraction yield of FBH using $scCO_2$ + EtOAc at different EtOAc to solids mass ratio (MR), for both cases, temperature presented a positive effect over the extraction yield for extractions at (1:1) MR, and a marginal negative effect at (2:1) MR. On the other hand, pressure presented a negative effect on the extraction yield for both

EtOAc to solids mass ratio. Meantime, the interaction between the two factors was positive for both ratios of cosolvent (MR).

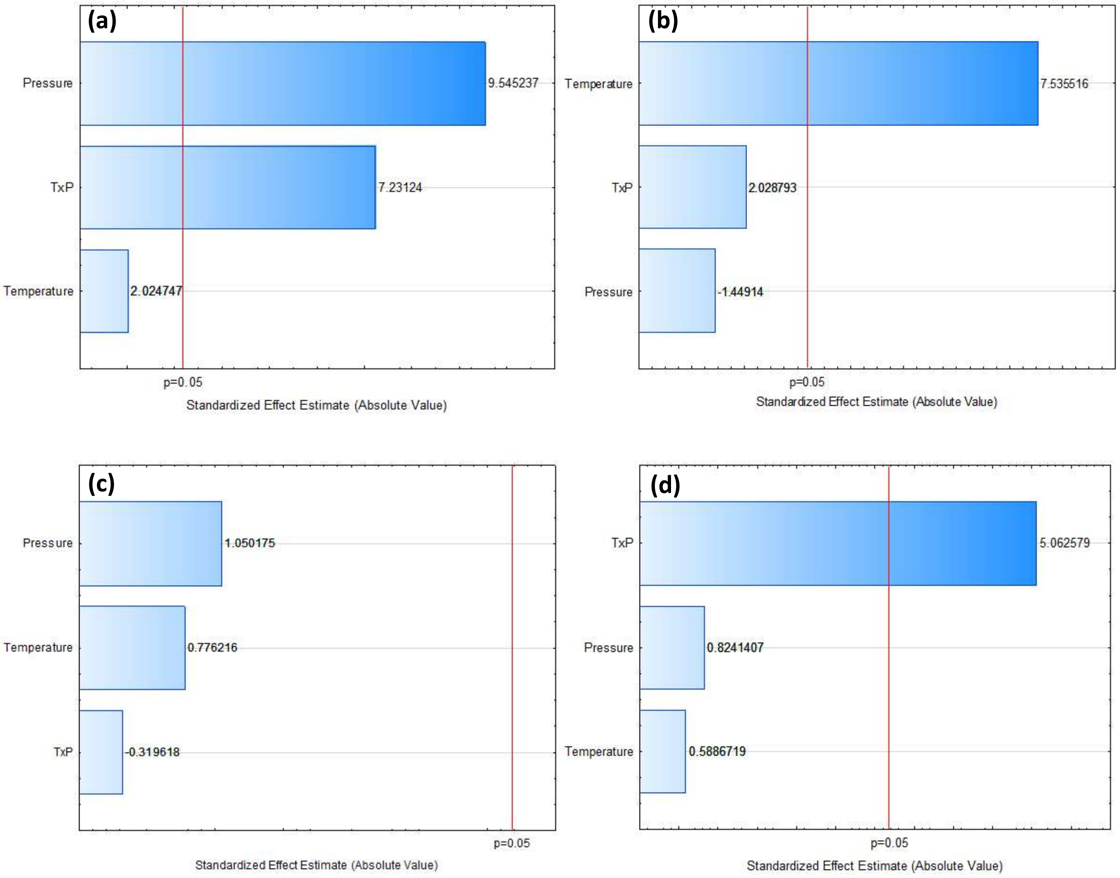


Figure 1 - Pareto chart of standardized effects over the extraction yield of **(a)** $scCO_2$; **(b)** propane; **(c)** $scCO_2$ + EtOAc at MR (1:1), and **(d)** $scCO_2$ + EtOAc at MR (2:1).

Table 2 – Overall extraction yields of FBH using SFE, PFE, $scCO_2$+cosolvent.

Run	Extraction Method	T (°C)	P (MPa)	MR[c]	Extraction time (min)	Extraction Yield (wt%)	Extraction efficiency (%)[d]	S/F[e]	Total compressed solvent (g)[f]
1	**SFE** ($scCO_2$)	40	15	-	110	5.6	82	8.2	251
2		40	25		110	6.0	89	6.3	228
3		80	15		110	4.7	70	4.4	248
4		80	25		110	**7.6**	113	5.6	214
5, 6, 7		60	20		110	6.2 ± 0.1	92	6.1 ± 0.1	205 ± 5
8	**PFE** (Propane)	20	3	-	70	3.9	58	4.7	111
9		20	10		70	2.7	40	5.2	118
10		60	3		70	5.8	87	4.7	153
11		60	10		70	**6.0**	90	4.5	110
12, 13, 14		40	6.5		70	4.2 ± 0.2	63	4.5 ± 0.2	107 ± 6
15	($scCO_2$ + EtOAc)	40	15	2:1	60	9.6	84	8.5	167
16		40	25	2:1	60	7.8	68	8.5	177
17		80	15	2:1	60	7.7	68	12.4	162
18		80	25	2:1	60	**10.2**	89	6.1	107
19, 20, 21		60	20	2:1	60	8.3 ± 0.2	73	6.1 ± 0.9	126 ± 2
22		40	15	1:1	60	5.6	49	6.7	189
23		40	25	1:1	60	7.1	63	7.1	181
24		80	15	1:1	60	6.8	60	4.3	154
25		80	25	1:1	60	7.6	67	5.9	171
26, 27, 28		60	20	1:1	60	**8.1** ± 0.2	71	5.9 ± 0.2	171 ± 5
29	($scCO_2$ + EtOH)	40	15	2:1	30	9.4	36	8.4	123
30		80	25	2:1	30	**10.5**	41	8.0	109
31		60	20	2:1	30	8.7	34	6.7	107

[c] Mass Ratio = Mass of cosolvent (g) / mass of FBH (g) at the initial time of dynamic extraction (t = 0 min).

[d] Extraction efficiency = Yield of pressurized extraction / yield of Soxhlet extraction. SFE and PFE were compared to n-hexane, $scCO_2$ + EtOAc was compared to EtOAc, and $scCO_2$ + EtOH compared to EtOH.

[e] Solvent to raw material (FBH)o (wt/wt), i.e., the mass of solvent injected to the extraction vessel to pressurize the system in g of solvent/mass of FBH (g) at the initial time of static extraction. For SFE and $scCO_2$+cosolvent, the solvent is $scCO_2$, and for PFE, the solvent is propane.

[f] Total solvent consumed during the extraction. i.e., the mass of solvent injected into the vessel to pressurize the system + solvent consumed within the total dynamic extraction. For SFE and $scCO_2$+cosolvent, the solvent is $scCO_2$, and for PFE the solvent is propane.

The extraction yield for $scCO_2$ + EtOAc at (1:1) MR showed a maximum at the central point (8.1%, at 20 MPa and 60 ºC, see Table 2), while for extractions at (2:1), the maximum yield (10.2%) was obtained at the highest condition of pressure and temperature (25 MPa and 80 ºC). Comparing the results in Table 2 for extraction using $scCO_2$ + EtOAc for the two different EtOAc to solids mass ratios, (2:1) and (1:1), the overall extraction yields were similar when the same pressure and temperature condition was used, except at 25 MPa and 80 ºC, where the maximum yield was obtained at (2:1). It is worth mentioning that 25 MPa and 80 ºC was the condition that led to the highest extraction yield when using pure $scCO_2$. Still, comparing the extraction using $scCO_2$ + EtOAc with $scCO_2$ + ethanol (Table 2), the results were statistically similar, indicating no difference in the extraction process using an alcohol or an ester as a cosolvent. These results reinforce the idea that the $scCO_2$ is the leading agent for the extraction process of this raw material and the liquid solvent (either ethyl acetate or ethanol) works to increase the solubility of the solutes in $scCO_2$, increasing the extraction rates and getting higher extraction performance.

For all cases, except by PFE, the overall extraction yield was increased by increasing the temperature and pressure. For compressed propane, only the temperature presented a positive effect on the extraction yield, since increasing the pressure, in a subcritical state at a constant temperature, propane in the liquid state has its diffusion into the matrix compromised by the increase in its density and viscosity.

Two main reasons for the temperature effect over the overall extraction yield performance in an isobaric process are the density of the solvent and the vapor pressure of the solute. As increasing the temperature increases the vapor pressure, an increase in the solute solubility in the solvent occurs. On the other hand, increasing the temperature, the density of the solvent decreases, reducing the solvation capacity [42]. Due to this effect, extractions at high temperature and high pressure resulted in higher overall yields.

The results presented in this study have shown that the application of cosolvent improves the extraction yield by increasing the solubility of polar compounds present in the hops matrix, but it can be a factor in decreasing the selectivity of the extraction [43].

3.1.1. Overall extraction curves

Figure 2 depicts the overall extraction curves for extraction using $scCO_2$ and propane. It can be observed that the highest initial extraction rate and highest extraction yield for the SFE were obtained at the highest temperature and pressure levels (Run 4) investigated in this work (80 °C, 25 MPa), followed by other conditions at 40 °C and then at the central point. All these SFE curves presented the three extraction periods, named constant extraction rate (CER), falling extraction rate (FER), and diffusion-controlled period (DCP). These results observed for hop extractions (Figure 2a) are indicating that the density of the supercritical fluid is the main parameter driving the extraction. The temperature of the process is an important factor that is increasing the vapor pressure of solutes, the diffusivity of CO_2, and increasing the solubility of compounds in dense $scCO_2$ medium, leading to high initial extraction rates.

For the extractions using compressed propane as the solvent, the initial extraction rates were similar, indicating that the solubility of hop compounds in compressed propane is, possibly, invariant within the range of temperature and pressure investigated. However, the overall extraction yield is statistically significant with the increase in the temperature, as already discussed.

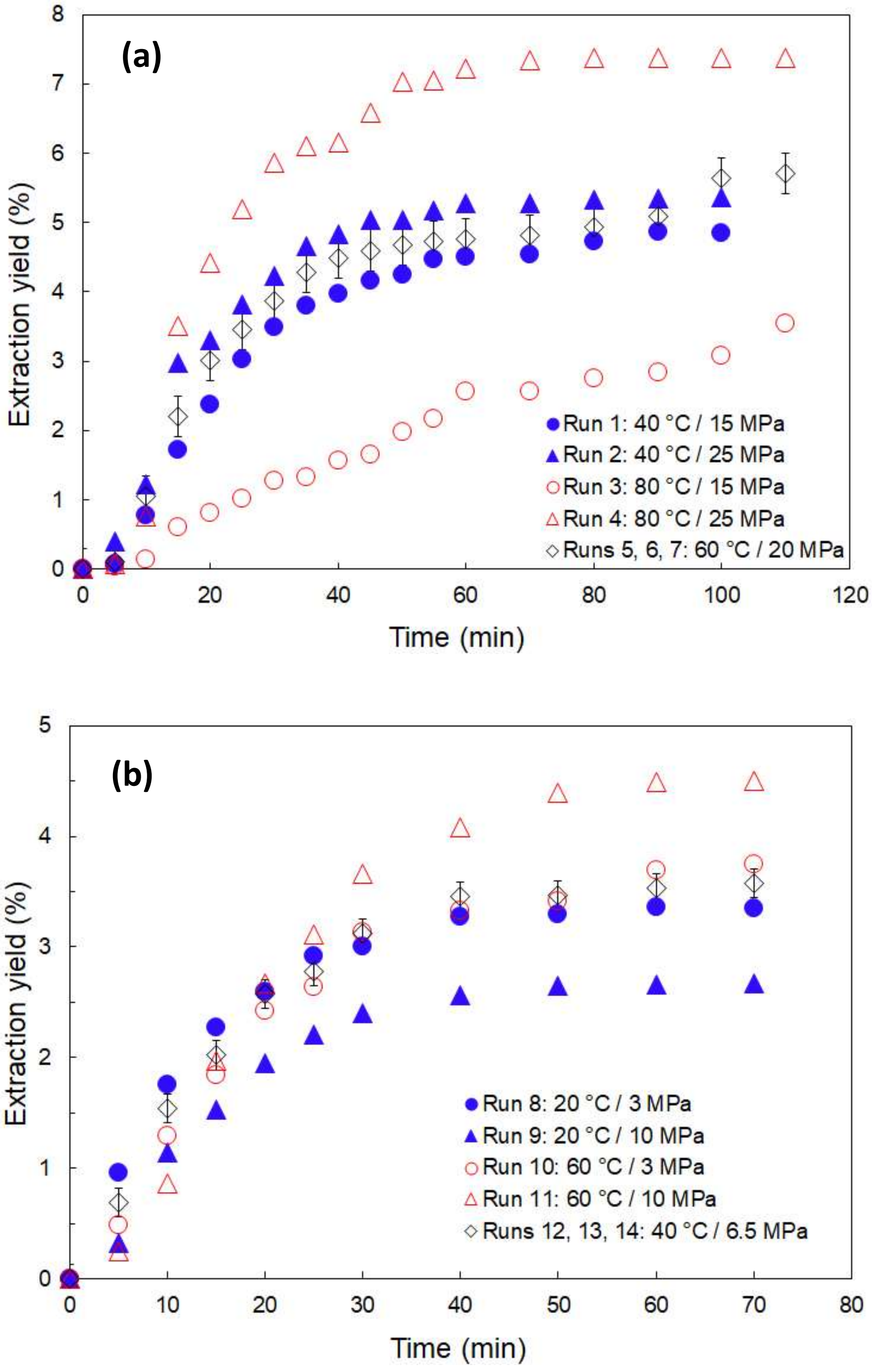
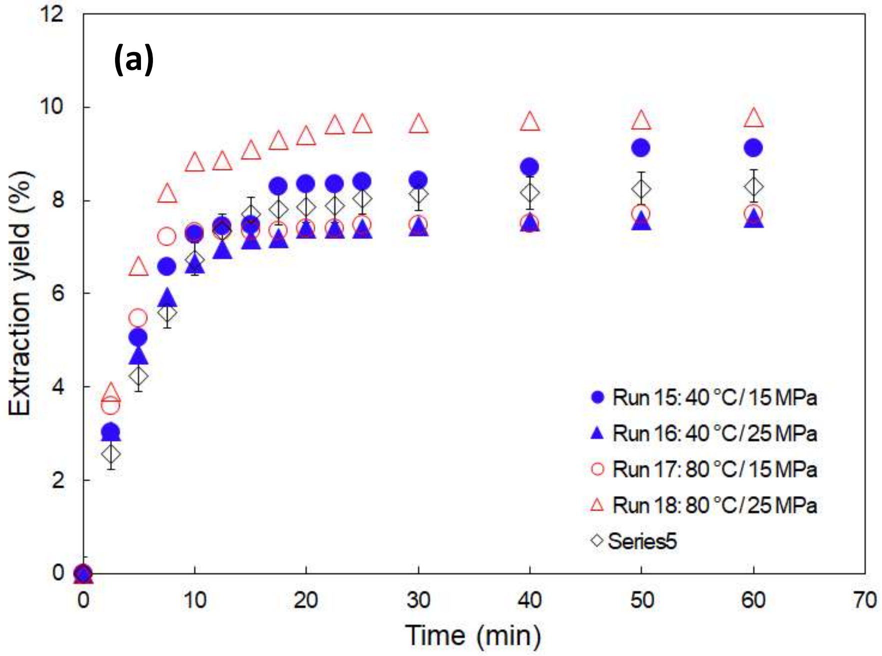


Figure 2 – Overall extraction curves of FBH using **(a)** $scCO_2$ and **(b)** propane as the solvent.

Figure 3 presents the overall extraction curves for extraction using $scCO_2$ + EtOAc. As presented in Figure 3(a), for the extraction with ethyl acetate (EtOAc) to FBH mass ratio of (2:1), the highest extraction rates were obtained at 80 °C and 15 MPa (Run

17) and 25 MPa. However, even presenting similar initial extraction rates, the condition at 80 °C and 25 MPa (Run 18) presented a final higher extraction yield when compared to 15 MPa. At 40 ºC, an opposite effect of pressure is observed, increasing the pressure from 15 MPa to 25 MPa the initial extraction rates were almost the same but decreased the yield of the extraction at the end of the process. It is possible to see a strong correlation between the temperature and the initial extraction rate and mainly on the overall extraction yield. If the extraction is conducted at constant pressure, both the solubility and diffusivity are improved by increasing the temperature, making the static extraction more effective [18]. It is worth mentioning that for the results in Figure 3(a), all extraction conditions presented a constant extraction rate until 15 min, approximately, and then jumped to a diffusion-controlled period. Figure 3(b) presents the extraction curves for conditions with EtOAc to FBH mass ratio of (1:1) (MR), and differently of extractions at (2:1), this condition at lower cosolvent related to the biomass, presented the usual three steps extraction, i.e., after a constant extraction rate (CER) period, the process went through a falling extraction rate (FER) and then a diffusive controlled period (DCP). The transition between CER and FER occurred around minute 10, and the transition to DCP around minute 25. For the results presented in Figure 3(b), the highest initial extraction rate was observed at 80 °C and 15 MPa (Run 24). Nevertheless, after 17.5 min, the condition at the central point (60 °C and 20 MPa) provided higher extraction yields. It is indicating that a lower initial amount of liquid solvent (ethyl acetate) the density of CO_2 has an important role in the kinetics of this extraction, and the same extraction performance is obtained for extraction at 80 ºC and 15 MPa (or 25 MPa) and 60 ºC and 20 MPa.



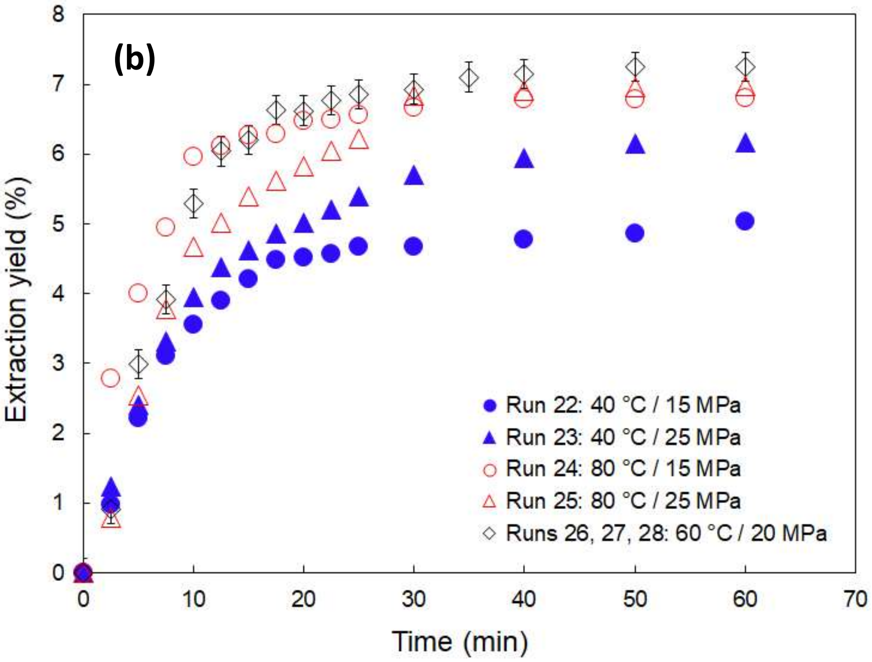


Figure 3 – Overall extraction curves for $scCO_2$ + EtOAc at different EtOAc to solids mass ratio: **(a)** (2:1) and **(b)** (1:1).

From the results presented in Figure 3(a), it was observed that after 15 min of extraction, the cosolvent was totally carried by the CO_2 from the extraction vessel, and then, after this point, the extraction was performed only with CO_2 being fresh pumped into the extractor. Figure 4 is qualitatively showing the ethyl acetate profile inside the extractor during different extraction conditions with $scCO_2$+EtOAc of temperature, pressure, and solvent to solids mass ratio. For extractions at a solvent to solids mass ratio (2:1), after an extraction time around 20 min, the liquid solvent is almost totally removed from the extraction vessel by fresh $scCO_2$. On the other hand, for extractions at (1:1), the liquid cosolvent extraction rate is slower than at (2:1), and ethyl acetate is removed from the extraction vessel only around 60 min of extraction. These ethyl acetate extractions by fresh $scCO_2$ were also visually observed during the extractions by the absence of liquid solvent collected in the flasks during the extraction sampling after ~20 min and ~50 min for experiments at (2:1) and (1:1) of MR, respectively. Therefore, it can be seen that in this semi-batch extraction approach, when the cosolvent is removed from the extraction vessel, the extraction is set back to a pure CO_2 extraction condition, which means that this type of extraction could be stopped at 10 to 15 min of extraction, where the maximum extraction rate and yield were observed. Thus, the results presented in this work are showing that it is possible to perform the extraction of hops using $scCO_2$ plus ethyl acetate as cosolvent, obtaining higher extraction yields in shorter times of process, comparing to the extraction with pure $scCO_2$.

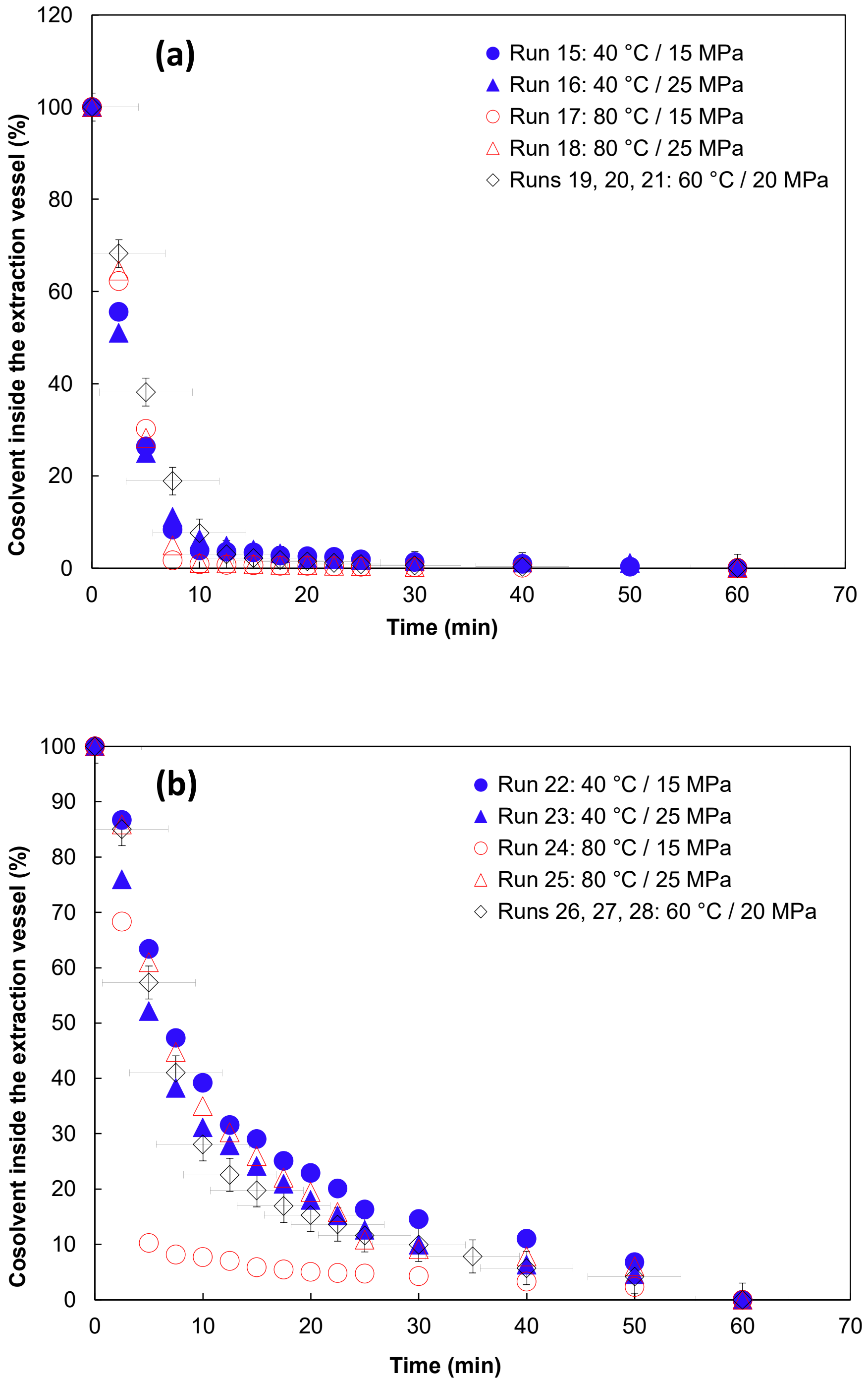


Figure 4 - Ethyl acetate (wt%) remaining inside the extractor vessel (calculated by mass balance) in semi-batch scCO$_2$+ethyl acetate extractions at cosolvent to solids mass ratio (MR) of **(a)** (2:1) and **(b)** (1:1).

3.2. Total phenolic content, total flavonoid content, and antioxidant activity

The results for the assays of TPC, TFC, and AA are presented in Table 3. All extracts obtained from FBH presented considerable TPC values varying between 87 mg GAE.g^{-1} and 189 mg GAE.g^{-1}. These values are 20 times higher than previous studies with grape using similar techniques [44,45]. These high values can be a combination of different factors like the pretreatment (freeze-drying) of the raw material, storage conditions, maturation stage, and growing conditions, in addition to differences between extraction conditions.

The highest value of TPC was found in the extract obtained by SFE with CO_2. All extracts of this group of extraction presented high TPC with values comprehended in the range 175 – 189 mg GAE.g^{-1}. The extract with the maximum TPC was obtained at 80 °C and 25 MPa (Run 4). Even in higher temperature extractions, the phenolic compounds are preserved.

The TPC values of the FBH extracts obtained by PFE with propane as a solvent were independent of extraction conditions. These extracts showed similar TPC, comprehended between 123 and 138 mg GAE.g^{-1}, and although these values are promising, they are lower than the extracts obtained by SFE and SFE + EtOAc.

Ethyl acetate, combined with $scCO_2$, was shown to be an excellent solvent for phenolic compounds. All extracts obtained using $scCO_2$ + EtOAc presented high TPC values, comprehended between 150 and 181 mg GAE.g^{-1}. Temperature and pressure conditions did not present significant differences in the outcome of the TPC. However, there is a tendency to a higher concentration of phenolics in extracts obtained at lower temperatures and MR = 2:1.

Extracts obtained by $scCO_2$ + EtOH presented similar values for TPC in the range between 87 and 91 mg GAE.g^{-1}, presenting the lowest results for TPC comparing with results obtained using ethyl acetate as cosolvent. This result was not expected considering the polarity of ethanol and, consequently, the affinity with phenolic compounds compared to ethyl acetate. However, the use of ethanol in $scCO_2$ extractions may favor the extraction of other compounds from the hops matrix, such as carbohydrates, reducing the concentration of phenolics in the final extract. Due to this lack of selectivity, the TPC suffer dilution in these extracts (Table 2). When comparing the overall yields of exhaustive extractions (Table 1), in which the yield with ethanol was 2.3 times higher than that of ethyl acetate, it is indicating that each solvent extracted different classes and fractions of compounds.

The analysis of TFC presented the tendency of an increase in values for lower temperatures of extraction using $scCO_2$. For the PFE, the increase of temperature induce a reduction of TFC as well, and the increase of pressure enhanced the extraction of flavonoids. The FBH extracts with the highest TFC were obtained using the only $scCO_2$, followed by the extracts obtained using $scCO_2$ + EtOAc that presents similar values independently of conditions of extraction (temperature, pressure, or MR). TFC values for extracts of these two methods are ranged from 60 up to 93 mg CE.g^{-1} and 51 up to 70 mg CE.g^{-1}, respectively.

Once more, the ethyl acetate as a cosolvent produces a richer in flavonoids extract when compared to the ethanol as a cosolvent. However, in opposition to the TPC results, the extracts obtained by $scCO_2$ + EtOH presented higher TFC (35 – 42 mg CE.g^{-1}) than extracts obtained by PFE (18 – 30 mg CE.g^{-1}), ratifying the higher affinity of ethanol by different flavonoids.

All extracts have high antioxidant activity values even when compared to several different matrices [46], the values ranging from 0.60 to 1.53 mmol TEAC.g^{-1} corroborating all the TPC and TFC analysis. The extracts with the highest values for antioxidant capacity were obtained by $scCO_2$ extractions, followed by $scCO_2$ + EtOAc. This result was expected considering the values of TPC and TFC. Moreover, hops are a source of phenolics compounds derived from benzoic and cinnamic acid, in addition to flavonoids that can retard or prevent oxidation, acting as radical scavengers.

Table 3 - Total phenolic content, total flavonoid content, and antioxidant activity by DPPH•

Run	**Extraction Method**	**T (°C)**	**P (MPa)**	**MR[c]**	**TPC (mg GAE.g^{-1})**	**TFC (mg CE.g^{-1})**	**DPPH• (mmol TEAC.g^{-1})**
1	SFE (CO2)	40	15	-	185 ± 9	**93 ± 4**	**1.53 ± 0.02**
2		40	25		176 ± 10	90 ± 5	1.46 ± 0.04
3		80	15		175 ± 8	64 ± 1	1.31 ± 0.04
4		80	25		**189 ± 4**	91 ± 2	1.35 ± 0.02
5, 6, 7		60	20		180 ± 13	60 ± 8	1.27 ± 0.03
8	PFE (Propane)	20	3	-	128 ± 2	25 ± 2	**1.15 ± 0.03**
9		20	10		128 ± 7	**30 ± 0**	1.10 ± 0.06
10		60	3		131 ± 4	18 ± 1	0.95 ± 0.03
11		60	10		**138 ± 4**	21 ± 1	1.05 ± 0.04
12, 13, 14		40	6,5		123 ± 5	23 ± 1	1.02 ± 0.08
15	$scCO_2$ + EtOAc	40	15	2:1	**181 ± 2**	57 ± 3	**1.27 ± 0.03**
16		40	25	2:1	162 ± 8	52 ± 8	1.17 ± 0.02
17		80	15	2:1	150 ± 16	58 ± 4	1.12 ± 0.03
18		80	25	2:1	156 ± 8	51 ± 3	1.14 ± 0.03
19, 20, 21		60	20	2:1	167 ± 20	**65 ± 9**	**1.27 ± 0.03**
22		40	15	1:1	174 ± 9	61 ± 3	**1.32 ± 0.04**
23		40	25	1:1	169 ± 21	**70 ± 4**	1.27 ± 0.01
24		80	15	1:1	164 ± 3	56 ± 1	1.18 ± 0.04
25		80	25	1:1	**176 ± 5**	59 ± 9	1.14 ± 0.04
26, 27, 28		60	20	1:1	**176 ± 5**	59 ± 1	1.23 ± 0.05
29	$scCO_2$ + EtOH	40	15	2:1	87 ± 6	**42 ± 2**	**0.62 ± 0.01**
30		80	25	2:1	**91 ± 9**	35 ± 1	0.60 ± 0.03
31		60	20	2:1	89 ± 3	39 ± 2	0.13 ± 0.04

[c] Mass Ratio = Mass of cosolvent (g) / mass of FBH (g) at the start of the extraction.

Conditions presented as Run 4, the best global yield for SFE, presented the best TPC and the second-best TFC. This same trend can be observed in Runs 25 and 30. Thus, it is possible to notice that the increase in yield does not compromise the quality of the extracts obtained from the FBH within the conditions evaluated in this study.

4. Conclusions

In this work, different non-conventional extractions were used to obtain extracts of hops (*Humulus lupulus*) Mantiqueira variety; the addition of ethyl acetate enhanced the extraction of global yield, rate, and efficiency. The best extraction was achieved using $scCO_2$ + EtOAc at RM (2:1) and 80 °C and 25 MPa, with a global yield of 10.2 wt%. The results indicated that in general terms, temperature and pressure combined have a positive effect on the extraction yield.

The results of TPC and TFC indicate that the use of EtOAc as a cosolvent does not reduce the quality of the extracts as antioxidant agents. The FBH extracts obtained using $scCO_2$ + EtOAc presented similar TPC when compared to pure $scCO_2$ extraction. Ethanol as cosolvent enhanced the overall extraction yield but presented a significant reduction in the selectivity for phenolic compounds. All the extracts presented a high antioxidant activity, expected effect due to the high concentrations of phenolic compounds found in the extracts from hops.

This study demonstrated that ethyl acetate is a promising cosolvent in $scCO_2$ extractions due to increased yield and phenolic compound extraction in various plant matrices. It is also possible to conclude that hops produced in Brazil are a rich source of bioactive compounds, even those produced under different climate conditions.

Brazilian hop extracts obtained by the extraction techniques presented in this work have great potential for use in the pharmaceutical and food industry, and a more detailed study of the composition of the extracts is suggested.

Acknowledgments

The authors would like to thank the Brazilian funding agencies (MLC thanks the CNPq, grant numbers 305393/2016-2, and 435873/2018-0 and Fundação Araucária – grant agreement 004/2019, APS is grateful for CNPq grant 305630/2018-0 and BAV thanks the CAPES, grant number 88881.361764/2019-01) for financial support and scholarships. ME and MLC also acknowledge the funding received from the European Union's Horizon 2020 research and innovation program under the Marie Sklodowska-Curie grant agreement No 778168.

Abbreviations

AA	Antioxidant activity
ANOVA	Analysis of variance
BH	Brazilian hops
CE	Catechin equivalent
CER	Constant extraction rate
DCP	Diffusive controlled period
DPPH	2,2-diphenyl-1-picrylhydrazyl
EtOAc	Ethyl acetate (Ethyl ethanoate)
EtOH	Ethanol
FBH	Freeze-dried Brazilian hops
FER	Falling extraction rate
GAE	Gallic acid equivalent
MR	The ratio of cosolvent mass to Freeze-dried Brazilian hops mass at start of extraction ($g_{cosolvent}/g_{FBH}$)
PFE	Pressurized fluid extraction
$scCO_2$	Supercritical carbon dioxide
SFE	Supercritical fluid extraction
TEAC	Trolox equivalents antioxidant capacity
TFC	Total flavonoids content
TPC	Total phenolics content
UV-VIS	Ultraviolet-Visible

5. References

[1] J. Palmer, How to Brew: Everything you need to know to brew beer right the, 1º ed, NATL Book Network, 2006.

[2] S. Lafontaine, S. Varnum, A. Roland, S. Delpech, L. Dagan, D. Vollmer, T. Kishimoto, T. Shellhammer, Impact of harvest maturity on the aroma characteristics and chemistry of Cascade hops used for dry-hopping, Food Chem. 278 (2019) 228–239. https://doi.org/10.1016/j.foodchem.2018.10.148.

[3] C. Schönberger, T. Kostelecky, The Role of Hops in Brewing, J. Inst. Brew. 117 (2011) 259–267. https://doi.org/10.1002/j.2050-0416.2011.tb00471.x.

[4] M. Biendl, Hops and Health, Tech. Q. 46 (2009). https://doi.org/10.1094/TQ-46-2-0416-01.

[5] A. Maietti, V. Brighenti, G. Bonetti, P. Tedeschi, F.P. Prencipe, S. Benvenuti, V. Brandolini, F. Pellati, Metabolite profiling of flavonols and in vitro antioxidant activity of young shoots of wild Humulus lupulus L. (hop), J. Pharm. Biomed. Anal. 142 (2017) 28–34. https://doi.org/10.1016/j.jpba.2017.04.043.

[6] L.R. Chadwiek, D. Nikolic, J.E. Burdette, C.R. Overk, J.L. Bolton, R.B. Van Breemen, R. Fröhlich, H.H.S. Fong, N.R. Farnsworth, G.F. Pauli, Estrogens and congeners from spent hops (Humulus lupulus), J. Nat. Prod. 67 (2004) 2024–2032. https://doi.org/10.1021/np049783i.

[7] S. Milligan, J. Kalita, V. Pocock, A. Heyerick, L. De Cooman, H. Rong, D. De Keukeleire, Oestrogenic activity of the hop phyto-oestrogen, 8-prenylnaringenin, Reproduction. 123 (2002) 235–242. https://doi.org/10.1530/rep.0.1230235.

[8] J. Bowe, X.F. Li, J. Kinsey-Jones, A. Heyerick, S. Brain, S. Milligan, K. O'Byrne, The hop phytoestrogen, 8-prenylnaringenin, reverses the ovariectomy-induced rise

in skin temperature in an animal model of menopausal hot flushes, J. Endocrinol. 191 (2006) 399–405. https://doi.org/10.1677/joe.1.06919.

[9] L. Bocquet, C. Rivière, C. Dermont, J. Samaillie, J.L. Hilbert, P. Halama, A. Siah, S. Sahpaz, Antifungal activity of hop extracts and compounds against the wheat pathogen Zymoseptoria tritici, Ind. Crops Prod. 122 (2018) 290–297. https://doi.org/10.1016/j.indcrop.2018.05.061.

[10] J.C. Lee, J.K. Kundu, D.M. Hwang, H.K. Na, Y.J. Surh, Humulone inhibits phorbol ester-induced COX-2 expression in mouse skin by blocking activation of NF-κB and AP-1: IκB kinase and c-Jun-N-terminal kinase as respective potential upstream targets, Carcinogenesis. 28 (2007) 1491–1498. https://doi.org/10.1093/carcin/bgm054.

[11] K. Yasukawa, A. Yamaguchi, J. Arita, S. Sakurai, A. Ikeda, M. Takido, Inhibitory effect of edible plant extracts on 12-O-tetradecanoylphorbol–13-acetate-induced ear oedema in mice, Phyther. Res. 7 (1993) 185–189. https://doi.org/10.1002/ptr.2650070218.

[12] L. Bravo, Polyphenols: Chemistry, Dietary Sources, Metabolism, and Nutritional Significance, Nutr. Rev. 56 (2009) 317–333. https://doi.org/10.1111/j.1753-4887.1998.tb01670.x.

[13] M.E. Embuscado, Spices and herbs: Natural sources of antioxidants – a mini review, J. Funct. Foods. 18 (2015) 811–819. https://doi.org/10.1016/J.JFF.2015.03.005.

[14] B. Pavlić, S. Vidović, J. Vladić, R. Radosavljević, Z. Zeković, Isolation of coriander (Coriandrum sativum L.) essential oil by green extractions versus traditional techniques, J. Supercrit. Fluids. 99 (2015) 23–28. https://doi.org/10.1016/J.SUPFLU.2015.01.029.

[15] A.D.P. do Nascimento, L.A.L. Soares, L. Stragevitch, L. Danielski, Extraction of Acrocomia intumescens Drude oil with supercritical carbon dioxide: Process modeling and comparison with organic solvent extractions, J. Supercrit. Fluids. 111 (2016) 1–7. https://doi.org/10.1016/j.supflu.2016.01.013.

[16] F. Sahena, I.S.M. Zaidul, S. Jinap, A.A. Karim, K.A. Abbas, N.A.N. Norulaini, A.K.M. Omar, Application of supercritical CO2 in lipid extraction – A review, J. Food Eng. 95 (2009) 240–253. https://doi.org/10.1016/J.JFOODENG.2009.06.026.

[17] S.C. Kupski, E.J. Klein, E.A. da Silva, F. Palú, R. Guirardello, M.G.A. Vieira, Mathematical modeling of supercritical CO2extraction of hops (Humulus lupulus L.), J. Supercrit. Fluids. 130 (2017) 347–356. https://doi.org/10.1016/j.supflu.2017.06.011.

[18] M.C. Mesomo, A.D.P. Scheer, E. Perez, P.M. Ndiaye, M.L. Corazza, Ginger (Zingiber officinale R.) extracts obtained using supercritical CO 2 and compressed propane: Kinetics and antioxidant activity evaluation, J. Supercrit. Fluids. 71 (2012) 102–109. https://doi.org/10.1016/j.supflu.2012.08.001.

[19] G.L. Teixeira, S.M. Ghazani, M.L. Corazza, A.G. Marangoni, R.H. Ribani, Assessment of subcritical propane, supercritical CO2 and Soxhlet extraction of oil from sapucaia (Lecythis pisonis) nuts, J. Supercrit. Fluids. 133 (2018) 122–132. https://doi.org/10.1016/j.supflu.2017.10.003.

[20] A.R. Guedes, A.R.C. de Souza, E.F. Zanoelo, M.L. Corazza, Extraction of citronella grass solutes with supercritical CO2, compressed propane and ethanol as cosolvent: Kinetics modeling and total phenolic assessment, J. Supercrit. Fluids. 137 (2018) 16–22. https://doi.org/10.1016/j.supflu.2018.03.003.

[21] M. Lanza, P.M. Ndiaye, F.W. Tavares, D. Oliveira, C. Dariva, J.V. Oliveira, Phase

behavior of castor oil in compressed propane and n-butane, J. Supercrit. Fluids. 34 (2005) 215–221. https://doi.org/10.1016/J.SUPFLU.2004.11.017.

[22] M. Correa, M.C. Mesomo, K.E. Pianoski, Y.R. Torres, M.L. Corazza, T.O.F. Contents, Extraction of inflorescences of Musa paradisiaca L. using supercritical CO2 and compressed propane, Adv. Account. 42 (2018) I–VIII. https://doi.org/10.1016/s0882-6110(18)30184-6.

[23] K. Czaikoski, M.C. Mesomo, A. de Paula Scheer, O.R. Dalla Santa, C.L. Queiroga, M.L. Corazza, Kinetics, composition and biological activity of Eupatorium intermedium flower extracts obtained from scCO2 and compressed propane, J. Supercrit. Fluids. 97 (2015) 145–153. https://doi.org/10.1016/J.SUPFLU.2014.10.008.

[24] M. Correa, M.C.M. Bombardelli, P.D. Fontana, F. Bovo, I.J. Messias-Reason, J.B.B. Maurer, M.L. Corazza, Bioactivity of extracts of Musa paradisiaca L. obtained with compressed propane and supercritical CO2, J. Supercrit. Fluids. 122 (2017) 63–69. https://doi.org/10.1016/j.supflu.2016.12.004.

[25] D.L. Fetzer, P.N. Cruz, F. Hamerski, M.L. Corazza, Extraction of baru (Dipteryx alata vogel) seed oil using compressed solvents technology, J. Supercrit. Fluids. 137 (2018) 23–33. https://doi.org/10.1016/j.supflu.2018.03.004.

[26] C.G. Pereira, M.A.A. Meireles, Supercritical Fluid Extraction of Bioactive Compounds: Fundamentals, Applications and Economic Perspectives, Food Bioprocess Technol. 3 (2010) 340–372. https://doi.org/10.1007/s11947-009-0263-2.

[27] M. Solana, I. Boschiero, S. Dall'Acqua, A. Bertucco, Extraction of bioactive enriched fractions from Eruca sativa leaves by supercritical CO_2 technology using different cosolvents, J. Supercrit. Fluids. 94 (2014) 245–251.

https://doi.org/10.1016/J.SUPFLU.2014.08.022.

[28] G. He, H. Xiong, Q. Chen, H. Ruan, Z. Wang, L. Traor&eacute. Optimization of conditions for supercritical fluid extraction of flavonoids from hops (*Humulus lupulus* L.), J. Zhejiang Univ. Sci. 6B (2005) 999–1004. https://doi.org/10.1631/jzus.2005.B0999.

[29] P.J. Magalhães, L.F. Guido, J.M. Cruz, A.A. Barros, Analysis of xanthohumol and isoxanthohumol in different hop products by liquid chromatography-diode array detection-electrospray ionization tandem mass spectrometry, J. Chromatogr. A. 1150 (2007) 295–301. https://doi.org/10.1016/j.chroma.2006.08.019.

[30] J.R. Edwardson, Hops: Their Botany, History, Production and Utilization. Published by: Springer on behalf of New York Botanical Garden Press Stable URL : https://www.jstor.org/stable/4252069 Hops-Their Botany, History, Production and Utilization, 6 (1952) 160–175.

[31] F. Van Opstaele, K. Goiris, G. De Rouck, G. Aerts, L. De Cooman, Production of novel varietal hop aromas by supercritical fluid extraction of hop pellets. Part 1: Preparation of single variety total hop essential oils and polar hop essences, Cerevisia. 37 (2013) 97–108. https://doi.org/10.1016/j.cervis.2012.12.002.

[32] V. Sanz, M.D. Torres, J.M. López Vilariño, H. Domínguez, What is new on the hop extraction?, Trends Food Sci. Technol. 93 (2019) 12–22. https://doi.org/10.1016/j.tifs.2019.08.018.

[33] M.N. Araújo, A.Q.P.L. Azevedo, F. Hamerski, F.A.P. Voll, M.L. Corazza, Enhanced extraction of spent coffee grounds oil using high-pressure CO2 plus ethanol solvents, Ind. Crops Prod. 141 (2019) 111723. https://doi.org/10.1016/j.indcrop.2019.111723.

[34] P. Trevisani Juchen, M. Nolasco Araujo, F. Hamerski, M.L. Corazza, F.A.

Pedersen Voll, Extraction of parboiled rice bran oil with supercritical CO2 and ethanol as cosolvent: Kinetics and characterization, Ind. Crops Prod. 139 (2019) 111506. https://doi.org/10.1016/j.indcrop.2019.111506.

[35] R. Coelho, L.R.S. Kanda, F. Hamerski, M.L. Masson, M.L. Corazza, Extraction of Kiwifruit Seed (Actinidia Deliciosa) Oil Using Compressed Propane, J. Food Process Eng. 39 (2016) 335–344. https://doi.org/10.1111/jfpe.12225.

[36] M. Correa, M.C. Mesomo, K.E. Pianoski, Y.R. Torres, M.L. Corazza, Extraction of inflorescences of Musa paradisiaca L. using supercritical CO2 and compressed propane, J. Supercrit. Fluids. 113 (2016) 128–135. https://doi.org/10.1016/j.supflu.2016.03.016.

[37] T. Fornari, Supercritical CO2 Extraction: Relevance to Food Processing, Elsevier, 2016. https://doi.org/10.1016/B978-0-08-100596-5.03111-5.

[38] V.L. Singleton, R. Orthofer, R.M. Lamuela-Raventós, [14] Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent, in: Methods Enzymol., Academic Press, 1999: p. 152–178. https://doi.org/10.1016/S0076-6879(99)99017-1.

[39] J. Zhishen, T. Mengcheng, W. Jianming, The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals, Food Chem. 64 (1999) 555–559. https://doi.org/10.1016/S0308-8146(98)00102-2.

[40] W. Brand-Williams, M.E. Cuvelier, C. Berset, Use of a free radical method to evaluate antioxidant activity, LWT - Food Sci. Technol. 28 (1995) 25–30. https://doi.org/10.1016/S0023-6438(95)80008-5.

[41] A.R. Guedes, A.R.C. de Souza, R.C. Turola Barbi, E.L. Nottar Escobar, É.F. Zanoello, M.L. Corazza, Extraction of Synadenium grantii Hook f. using conventional solvents and supercritical CO2 + ethanol, J. Supercrit. Fluids. 160

(2020) 8–10. https://doi.org/10.1016/j.supflu.2020.104796.

[42] M. Careri, L. Furlattini, A. Mangia, M. Musci, E. Anklam, A. Theobald, C. Von Holst, Supercritical fluid extraction for liquid chromatographic determination of carotenoids in Spirulina Pacifica algae: A chemometric approach, J. Chromatogr. A. 912 (2001) 61–71. https://doi.org/10.1016/S0021-9673(01)00545-3.

[43] M. HERRERO, A. CIFUENTES, E. IBANEZ, Sub- and supercritical fluid extraction of functional ingredients from different natural sources: Plants, food-by-products, algae and microalgae: A review, Food Chem. 98 (2006) 136–148. https://doi.org/10.1016/j.foodchem.2005.05.058.

[44] C. Da Porto, D. Decorti, A. Natolino, Water and ethanol as cosolvent in supercritical fluid extraction of proanthocyanidins from grape marc: A comparison and a proposal, J. Supercrit. Fluids. 87 (2014) 1–8. https://doi.org/10.1016/J.SUPFLU.2013.12.019.

[45] A. Natolino, C. Da Porto, S. Rodríguez-Rojo, T. Moreno, M.J. Cocero, Supercritical antisolvent precipitation of polyphenols from grape marc extract, J. Supercrit. Fluids. 118 (2016) 54–63. https://doi.org/10.1016/j.supflu.2016.07.015.

[46] D. Krishnaiah, R. Sarbatly, R. Nithyanandam, A review of the antioxidant potential of medicinal plant species, Food Bioprod. Process. 89 (2011) 217–233. https://doi.org/10.1016/J.FBP.2010.04.008.