Received: 05/12/2023 Revised: 14/01/2025 Accepted: 07/03/2025 Published: 24/04/2025

Journal of the Institute of Brewing
EST 1895

ORIGINAL ARTICLE

DOI 10.58430/jib.v131i1.69

Brazilian Mantiqueira hops: composition and potential industrial use

- Bruno A Veiga [1]
- Maria C Roda-Serrat [2]
- Mathias P Clausen [2]
- Massimiliano Errico [2]
- Marcos L Corazza [1]
- Agnes P Scheer [1]

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

[2] Department of Green Technology, University of Southern Denmark, Campusvej 55, 5230 Odense M, Denmark.

agnesps@ufpr.br

Abstract

Why was the work done: The global cultivation of hop varieties has expanded to Australia and South America, where the environmental conditions influence their composition. A Brazilian hop variety, Mantiqueira has potential applications in brewing, but the chemical composition and industrial suitability remain unexplored.
What are the main findings: The composition of Mantiqueira hops was determined by extracting the bioactive compounds using three pressurised fluid techniques: supercritical CO_2 ($scCO_2$), pressurised propane, and a pressurised mixture of $scCO_2$ and ethyl acetate or ethanol. High-performance liquid chromatography was used to identify and quantify alpha acids (humulone, cohumulone, and adhumulone), beta acids (lupulone, colupulone, and adlupulone), and the key phenolic compound xanthohumol.
Why is the work important: Supercritical CO_2 achieved the highest recovery of alpha and beta acids, with the seven compounds representing up to 98.9% of the extract mass. Pressurised propane selectively extracted alpha and beta acids, producing (potential) bitterness enhancing extracts with minimal phenolic content. The addition of ethyl acetate to $scCO_2$ as a co-solvent increased the xanthohumol concentration by over 30-fold compared to $scCO_2$ alone. Further, the combination of $scCO_2$ and ethanol enhanced the extraction of both (potential) bitter acids and bioactive compounds, with higher temperatures and pressures yielding improved efficiency and diversity of compounds. The chemical profile of Mantiqueira hops was comparable to that of the noble Czech and North American aroma hops.
Why is the work important: This study characterises a Brazilian hop variety with diverse applications. The findings report sustainable extraction methods and the composition of Mantiqueira hops, which could enhance beer flavour, aroma, and quality. Additionally, the high xanthohumol content supports the use of these hops in functional foods and nutraceuticals, broadening their industrial relevance and contributing to global hop diversification.

Keywords

bittering compounds; alpha acids; beta acids; xanthohumol; pressurised fluid extraction; co-solvent.

Introduction

Hops - *Humulus lupulus* - is a versatile plant recognised for its inflorescences (cones), which play a crucial role in beer production. While hops are primarily associated with brewing, their bioactive compounds - alpha acids, beta acids, and xanthohumol - offer potential applications in industries beyond brewing (Karabín et al. 2016; Bocquet et al. 2018). Historically, the largest producers of hops have been in the temperate regions of the Northern Hemisphere, particularly Germany, England, the Czech Republic, and the United States (Teghtmeyer 2018). In recent years, emerging hop-producing regions, such as Australia, Argentina, and Brazil, have gained attention through their cultivation conditions and increasing yields (Jastrombek et al. 2022; Herkenhoff et al. 2024).

The brewing industry depends on hops as a source of bittering compounds, primarily isomerised alpha acids (humulones, cohumulones, and adhumulones), which provide characteristic flavour and, to a degree natural, preservatives (Dresel et al. 2016). Beta acids (lupulones, colupulones, and adlupulones) contribute similarly, but to a lesser extent. The flavour and aroma profiles of hops are influenced by their essential oils and phenolic compounds, including xanthohumol, rutin, and proanthocyanidins (Goiris et al. 2014; Dias et al. 2024). Among these, xanthohumol, a prenylated flavonoid, has gained interest for its antibacterial, antioxidant, anti-inflammatory, and anticarcinogenic properties (Lupinacci et al. 2009; Liu et al. 2015; Bolton et al. 2019).

With the growth in the size and diversity of breweries, the demand for hops with distinctive sensory and chemical profiles has increased. Emerging hop-growing regions, such as South America, provide an opportunity to explore novel hop varieties with an interesting composition. Mantiqueira hops, a variety adapted to the Brazilian climate and derived from a mutation of an American hop (Cascade), represent a cultivar with commercial promise. Preliminary studies suggest that the chemical composition of Mantiqueira is comparable to the long established noble and aroma hops, making it suitable for both brewing and other applications (Veiga et al. 2021).

Recent advances in extraction technologies, particularly supercritical CO_2 ($scCO_2$), have revolutionised the recovery of bioactive compounds from hops, enabling the efficient isolation of alpha/beta acids and xanthohumol (Rój et al. 2015; Veiga et al. 2021). The incorporation of organic co-solvents with $scCO_2$ has further enhanced these processes, increasing the yields of phenolic compounds, which are sought after for functional and nutraceutical applications (Arruda et al. 2022; Sanz et al. 2022). Additionally, innovation in extraction methodologies have facilitated detailed chemical profiling of hop extracts and essential oils, expanding the identification of bioactive compounds with diverse applications in the brewing, food, and pharmaceutical industries (Duarte et al. 2023; Metaj et al. 2023; Paniagua-García et al. 2023; Kljakić et al. 2024).

The chemical composition and extraction efficiency of Brazilian hop varieties remains unexplored. Accordingly, this study investigates the chemical composition of Mantiqueira hops and evaluates the efficacy of different pressurised fluid extraction techniques in isolating key alpha/beta acids and bioactive compounds. By characterising the extracts and the concentration of alpha acids, beta acids, and xanthohumol, the research seeks to assess the potential application of Mantiqueira hops for brewing and other industrial applications.

Materials and methods

Formic acid (98% purity) was purchased from Sigma-Aldrich (Søborg, Denmark), acetonitrile and methanol (chromatographic purity) from VWR Prolabo (Søborg, Denmark). Deionised water (Milli-Q standard) was produced using an ELGA PureLab® Chorus system (Glostrup, Denmark).

The female flower extracts of Mantiqueira hops were analysed. Pressurised fluid-based extractions were used (Veiga et al. 2021), with three distinct extraction blocks. The first block used a fixed bed of hops extracted by pure supercritical carbon dioxide ($scCO_2$) under varying temperature and pressures. The second block used pressurised propane at different pressures and temperature. In the third block, hops were extracted using a mixture of $scCO_2$

and a green solvent (ethyl acetate or ethanol) at different concentrations, pressure and temperatures. The extraction parameters for each method are summarised in Table 1, with detailed descriptions found in Veiga et al (2021).

To assess extraction efficiency (EE), hop flowers and extracts were processed using a methanol-based solution to isolate phenolic compounds, using the methodology of Overk et al (2008). The solvent of 75% (v/v) methanol, 1% (v/v) formic acid, and 24% (v/v) water was used across the extraction process to enable HPLC quantification.

Hop sample preparation

Lyophilised hop flowers were weighed (0.5014 ± 0.0003 g) and combined with 15 mL of the methanol/formic acid/water solvent in a Falcon tube. The mixture was agitated and milled using the Ultra Turrax (IKA, Aarhus, Denmark) for 2 min. The instrument was rinsed with 5 mL of solvent, which was added back to the Falcon tube. An additional 30 mL of solvent was introduced in two steps, with agitation and milling on each addition for 2 min. The mixture was vortexed for 1 min and placed in an ultrasonic bath at 35°C for 10 min. The solid and liquid phases were separated by centrifugation at 25°C and 1500 × *g* for 20 min.

Table 1.

Summary of yields and extraction conditions for Mantiqueira hops.

Run	Solvent	T (°C)	P (MPa)	MR*	Extraction time (min)	Extraction Yield (% wt)
1	$scCO_2$	40	15	-	110	5.6
2		40	25		110	6.0
3		80	15		110	4.7
4		80	25		110	**7.6**
5, 6, 7		60	20		110	6.2 ± 0.1
8	Pressurised propane	20	3	-	70	3.9
9		20	10		70	2.7
10		60	3		70	5.8
11		60	10		70	**6.0**
12, 13, 14		40	6.5		70	4.2 ± 0.2
15	$scCO_2$ + EtOAc	40	15	2	60	9.6
16		40	25	2	60	7.8
17		80	15	2	60	7.7
18		80	25	2	60	**10.2**
19, 20, 21		60	20	2	60	8.3 ± 0.2
22		40	15	1	60	5.6
23		40	25	1	60	7.1
24		80	15	1	60	6.8
25		80	25	1	60	**7.6**
26, 27, 28		60	20	1	60	8.1 ± 0.2
29	$scCO_2$ + Ethanol	40	15	2	30	9.4
30		80	25	2	30	**10.5**
31		60	20	2	30	8.7

*MR = [mass of co-solvent at the start of extraction (g)] / [hop mass at the beginning of extraction (g)].

The supernatant was recovered in a 100 mL volumetric flask. The solid residue was treated with 15 mL of fresh solvent, with the agitation/milling and centrifugation step repeated twice. Finally, 10 mL of solvent solution was added to the remaining solid and subject to ultrasonication at 35°C for 15 min. The mixture was left at room temperature for 12 h, then vacuum filtered. The retained solid was rinsed with 15 mL of solvent solution, and the filtrate adjusted to 100 mL with solvent to obtain the methanol extract of lyophilised hop flowers.

Preparation of pressurised fluid-based extracts

Aliquots of pressurised fluid extracts (10.5 ± 0.2 mg) were dissolved in 2 mL of solvent solution in Eppendorf tubes. These were vortexed, sonicated (35°C, 20 min), centrifuged at 600 × *g*, and filtered through 0.22-µm PTFE syringe filters. The filtrates were transferred to amber vials for injection into the HPLC system.

Chemical composition by HPLC

High-performance liquid chromatography (HPLC) was used to quantify alpha acids (humulone, cohumulone, adhumulone), beta acids (lupulone, colupulone, adlupulone), and xanthohumol in Mantiqueira hops (Bessada et al 2016). The analysis used an HP 1200 series HPLC system (Agilent Technologies Aps, Nærum, Denmark) equipped with a photodiode array detector and a Gemini 5µm C18 110A column (250 × 4.6 mm i.d. Phenomenex Aps, Værløse, Denmark) maintained at 35°C.

The mobile phase comprised of solvent A (0.1% v/v aqueous formic acid) and solvent B (acetonitrile). A gradient elution was applied: 15% B for the first 5 min (isocratic), ramped to 20% B over 5 min, then 25% B over the next 10 min, 35% B over 10 min, and finally 50% B over 10 min, followed by re-equilibration. The flow rate was 0.5 mL/min, and detection was performed at 280, 320, and 370 nm. Retention times for the compounds were: xanthohumol (32 min), humulone (41 min), cohumulone (43 min), adhumulone (44 min), lupulone (50 min), colupulone (52 min), and adlupulone (53 min). Calibration standards were used achieving purities of 60.6-93.1%, exceeding ICE-3 standards. Calibration curves for each compound exhibited excellent linearity ($R^2 > 0.990$).

A preparative chromatography system (Dionex UltiMate 3000 Binary Semi-preparative LC; Thermo Fisher Scientific), equipped with a Develosil TM ODS-HG LC column (250 mm × 20 mm, 5 µm particle size), was used to separate seven standards with purities ranging from 60.6 to 93.1%. (These exceed the purity levels of the International Calibration Extract (ICE) 3 standard of the American Society of Brewing Chemists released in 2010. The ICE-3 standard comprises 44.6% humulone and 24.3% lupulone). The same solvent solutions (A and B) and gradient described for the analytical chromatographic method (above) were used for preparative chromatography, with adaptations made for the preparative equipment. The method produced consistent peak profiles between preparative and analytical chromatography. The samples were fractionated into nine individual components, which were subsequently analysed by HPLC to determine purity (Andrade 2018). The purity of each isolated compound was measured with values between 60.6 and 93.1%, which exceeded the levels found in the ICE-3 standard. These high purity fractions provided reliable standards for further analytical procedures and compound quantification.

Statistical analysis

The data were used to calculate the total yield, extraction efficiency (EE) for each component, and the purity of the compounds in the extracts. The total yield was determined using Equation 1:

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

The extraction efficiency (EE) for each component was calculated using Equation 2:

$$EE(\%) = \frac{\text{Mass of component } i \text{ extracted}}{\text{Mass of component } i \text{ on matrix}} \cdot 100\%$$

Analyses were conducted using Statistica 10 software (Statsoft Inc. USA). Response surface methodology (RSM) was used to assess the effects of temperature and pressure on the extraction of hop compounds, utilising quadratic polynomial equations to model the relationships between parameters and the extraction outcomes.

Results and discussion

As outlined in Table 1, the extracts were derived using different solvents, temperatures and pressure The molar fractions and extraction efficiencies of key compounds are reported in Table 2. These included xanthohumol, alpha acids (humulone, cohumulone, and adhumulone), and beta acids (lupulone, colupulone, and adlupulone), with extraction efficiency (EE) calculated according to Equation 2.

The antioxidant properties of Mantiqueira hop extracts have been attributed to their high concentration of phenolic compounds (Veiga et al. 2021).

The composition of Mantiqueira hops, from freeze-dried flower extracts, had a distinct profile (Figure 1). The concentration of alpha and beta acids in Mantiqueira hops were lower than those found in bittering hops such as Chinook, Cascade, and Nugget. However, the concentration of essential oil was comparable to Galaxy hops, which are renowned for their aromatic qualities (Machado et al. 2018). These findings suggest that Mantiqueira hops may not be ideal for bitterness but offer potential for flavour applications.

Extraction with supercritical CO_2 ($scCO_2$)

Extractions using $scCO_2$ (runs 1–7, Table 1) were conducted using a 2^2 factorial design with temperature and pressure as key variables, and with triplicates at the central point to evaluate the effects of temperature, pressure, and their interaction.

Table 2.

Extract composition and extraction efficiency for Mantiqueira hops by different pressurised techniques.

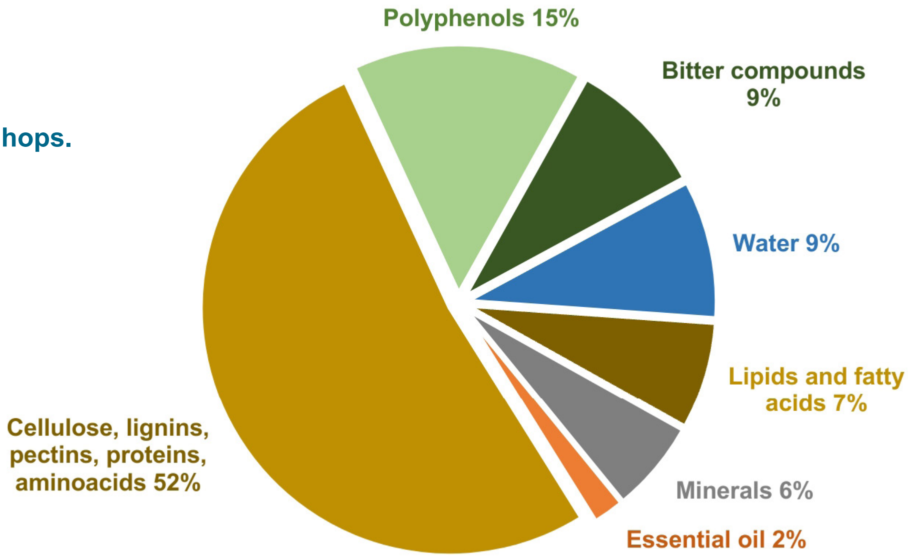
Run*	Sample mass ± SD (mg)	Known mass	Xanthohumol		Humulone		Cohumulone		Adhumulone		Lupulone		Colupulone		Adlupulone	
			MF	EE	MF	EE	MF	EE	MF	EE	MF	EE	MF	EE	MF	EE
1	0.0088 ± 0.0002	98.1	0.1	0.7	23.0	61.8	18.5	55.9	13.0	52.8	22.6	71.4	13.9	48.5	7.1	66.7
2	0.0109±0.0004	98.4	0.1	0.7	24.5	70.5	19.6	63.7	13.7	59.8	20.2	68.6	13.4	50.1	6.9	69.1
3	0.0115 ± 0.0003	93.6	0.00	0.0	18.9	42.7	16.8	42.7	13.1	44.8	20.0	41.7	16.6	30.9	8.1	43.1
4	0.0115 ± 0.0002	94.9	0.1	0.8	23.8	86.8	19.4	79.9	13.9	76.8	20.1	86.3	11.5	54.6	6.1	77.8
5, 6, 7	0.0107 ± 0.0002	98.9	0.1	0.3	22.7	67.5	18.8	63.1	13.9	62.7	21.0	73.5	14.8	57.5	7.7	79.4
8	0.0100 ± 0.0003	54.4	0.00	0.0	13.7	25.6	11.0	23.2	7.9	22.5	11.2	24.8	7.1	17.3	3.6	23.3
9	0.0106 ± 0.0005	80.8	0.00	0.0	20.0	25.9	15.5	22.7	11.5	22.7	16.6	25.4	11.4	19.3	5.8	26.3
10	0.0107 ± 0.0001	70.9	0.00	0.0	19.8	55.0	15.8	49.4	11.0	46.3	11.4	37.4	8.6	31.0	4.4	42.8
11	0.0106 ± 0.0002	71.3	0.00	0.0	18.9	54.4	15.5	50.1	10.7	46.6	12.5	42.5	9.0	33.8	4.7	47.1
12, 13, 14	0.0102 ± 0.0002	79.5	0.00	0.0	19.6	39.9	15.6	35.7	11.7	36.0	15.6	37.5	11.2	29.7	5.8	40.9
15	0.0106 ± 0.0003	92.6	5.1	48.5	23.7	109.4	18.8	97.8	13.9	97.3	15.8	86.0	10.0	60.0	5.2	83.4
16	0.0104 ± 0.0002	67.6	3.5	27.2	19.1	72.4	14.3	60.2	10.5	59.8	10.3	45.4	6.5	31.9	3.4	44.3
17	0.0106 ± 0.0001	84.9	5.5	41.9	21.4	79.0	17.3	72.1	13.0	72.8	13.8	60.2	9.1	43.8	4.8	61.9
18	0.0107 ± 0.0003	53.0	2.7	27.3	16.5	81.7	12.1	66.7	8.8	65.3	6.4	36.8	4.32	27.6	2.3	39.3
19, 20, 21	0.0106 ± 0.0003	79.3	5.3	44.1	21.0	83.5	17.0	76.3	12.6	76.2	11.5	54.2	7.72	40.0	4.1	57.0
22	0.0120 ± 0.0001	99.0	3.3	6.5	24.6	23.6	19.5	21.1	14.9	21.7	17.7	20.0	12.78	16.0	6.3	21.2
23	0.0106 ± 0.0001	92.4	5.1	35.3	24.1	80.3	19.0	71.5	13.7	69.6	14.6	57.3	10.47	45.5	5.5	63.8
24	0.0105 ± 0.0001	72.8	5.0	30.6	20.2	60.2	16.4	55.0	11.4	51.3	9.4	33.1	6.80	26.4	3.6	37.6
25	0.0106 ± 0.0004	75.9	3.5	25.6	21.9	76.3	17.5	69.0	12.3	65.0	10.0	41.0	7.10	32.4	3.8	45.7
26, 27, 28	0.0107 ± 0.0003	90.4	4.0	31.9	24.0	93.4	19.2	84.2	13.6	80.3	14.2	65.0	10.19	51.7	5.3	71.9
29	0.0105 ± 0.0003	24.4	3.7	34.1	6.0	26.8	5.1	25.7	3.8	25.8	3.7	19.6	1.59	9.3	0.7	11.4
30	0.0107 ± 0.0001	28.6	3.7	38.7	7.2	36.5	6.0	33.9	4.3	33.2	4.2	25.2	1.99	13.1	1.1	19.2
31	0.0103 ± 0.0002	26.6	3.8	33.3	23.0	61.8	18.5	55.9	13.0	52.8	3.5	17.3	1.59	8.6	1.0	14.4

All data as % weight. SD = standard deviation; MF = mass fraction; EE = extraction efficiency

***Runs 1 to 7** with supercritical CO_2, **runs 8 to 14** with pressurised propane, **runs 15 to 21** with supercritical CO_2 + ethyl acetate with MR=2, **runs 22 to 28** supercritical CO_2 + ethyl acetate with MR=1, and **runs 29 to 31** supercritical CO_2 + ethanol with MR=2. Details of the extraction conditions are presented in Table 1.

Figure 1.

Composition of Brazilian Mantiqueira hops.



The extraction yield was positively affected by both temperature and pressure, primarily due to the increased solubility of compounds in the solvent at higher temperatures. As the pressure increased, the fluid density increased, enhancing the solvation power (Rój et al. 2015; Pinto et al. 2023). However, no significant correlation between xanthohumol concentration and extraction efficiency was observed. In contrast, a combination of elevated temperature and pressure significantly improved the extraction efficiencies of the three alpha acids, as shown in the Pareto chart (Figure 2a). This suggests that pressure plays a critical role in optimising the extraction of these compounds. Because the extraction behaviour of the three alpha acids was comparable, only the response surface for humulone is shown in Figure 2b, the model of which disregarded the temperature variable. This behaviour aligns with the physical properties of $scCO_2$, where increased pressure enhances the fluid density, thereby improving the ability of the solvent to dissolve and extract hydrophobic compounds such as alpha acids (Klimek et al. 2021).

The extraction efficiencies of the beta acids (lupulone, colupulone, and adlupulone) were also evaluated using the same statistical approach. The results showed that while both temperature and pressure had statistically significant effects on lupulone extraction, the other beta acids were less responsive to the variations in these variables. The Pareto chart (Figure 2c) and response surface for lupulone (Figure 2d) showed that temperature had a minimal impact on its extraction efficiency, suggesting that pressure alone is a critical factor in extracting this compound. This finding is consistent with previous studies that demonstrated the advantages of $scCO_2$ extraction for maximising yield while maintaining the integrity of the extracted compounds (Zekovic et al. 2007; Rój et al. 2015; Pinto et al. 2023).

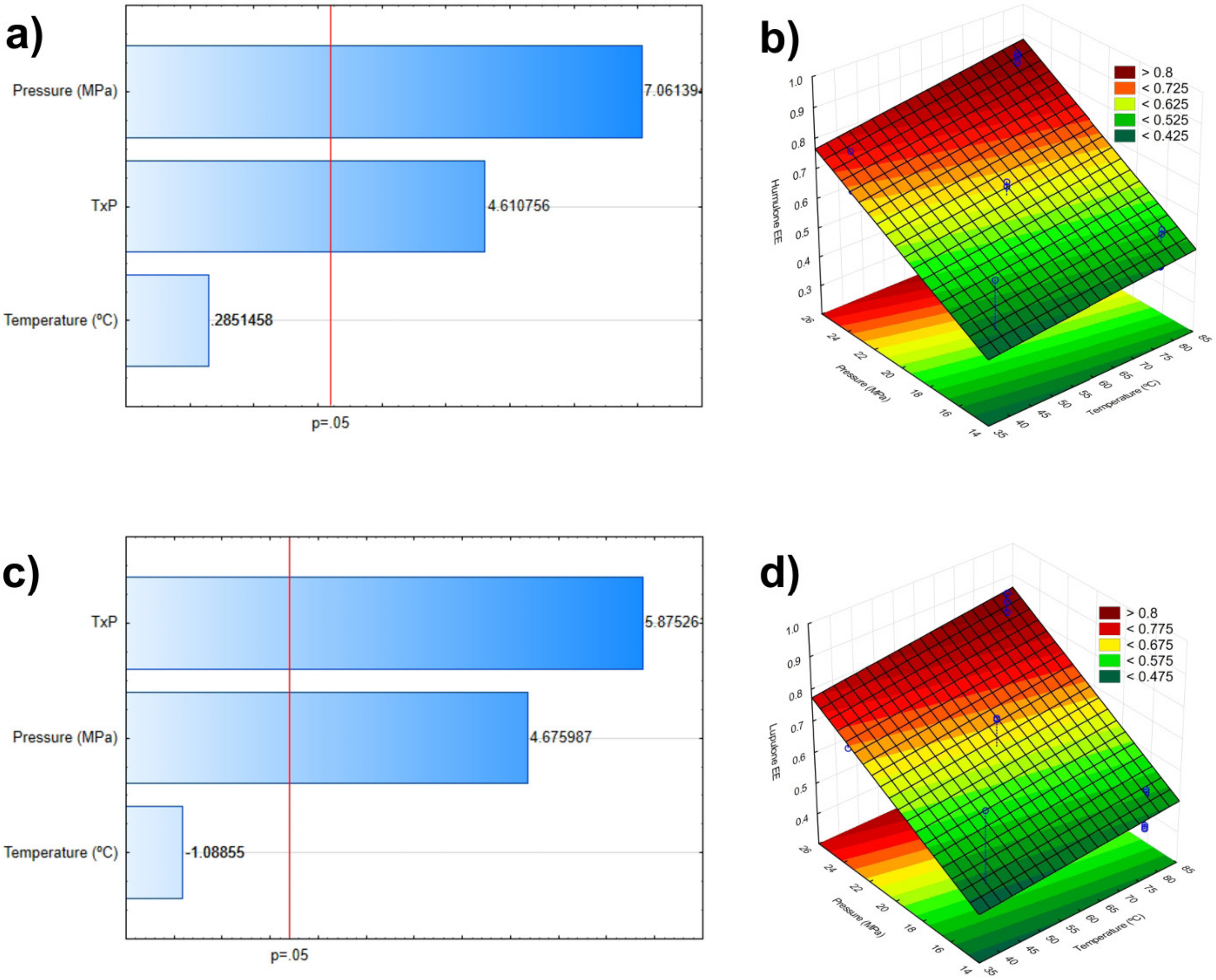
The overall results support the view that $scCO_2$ is a highly effective solvent for extracting bioactive compounds from hops, particularly alpha and beta acids, under optimised temperature and pressure conditions. The solvation power of $scCO_2$ is strongly influenced by pressure, which increases the fluid density and enhances its ability to dissolve a wide range of bioactive compounds. Moreover, the increase in temperature accelerates the rate of mass transfer from the solid matrix to the supercritical fluid (Pinto et al. 2023), improving the effeciency of extraction of compounds such as alpha acids.

Extraction with pressurised propane

The extractions using pressurised propane (runs 8–14, Table 1) followed a 2^2 factorial design like $scCO_2$. The results showed that the extraction yield increased significantly with temperature. This is consistent with other studies where higher temperatures enhance the solubility of compounds in nonpolar solvents, like propane (Bizaj et al. 2021). However, pressure variations within the range used here did not significantly influence the overall extraction yield, indicating that the density changes of propane at subcritical conditions had minimal impact on its solvation power.

Figure 2.

Pareto charts (a, c) and response surface plots (b, d) showing the effects of process parameters on the extraction efficiency (EE) of humulone and lupulone from Mantiqueira hops using supercritical CO$_2$. Extraction efficiency equations: $\text{EE}\ (\%) = 1.88 + 2.55\cdot P + 0.01\cdot PT$ for humulone, and $\text{EE}\ (\%) = 8.237 + 2.418\cdot P + 0.0068\cdot \text{PT}$ for lupulone, where *P* represents pressure, and *PT* represents the pressure-temperature interaction.



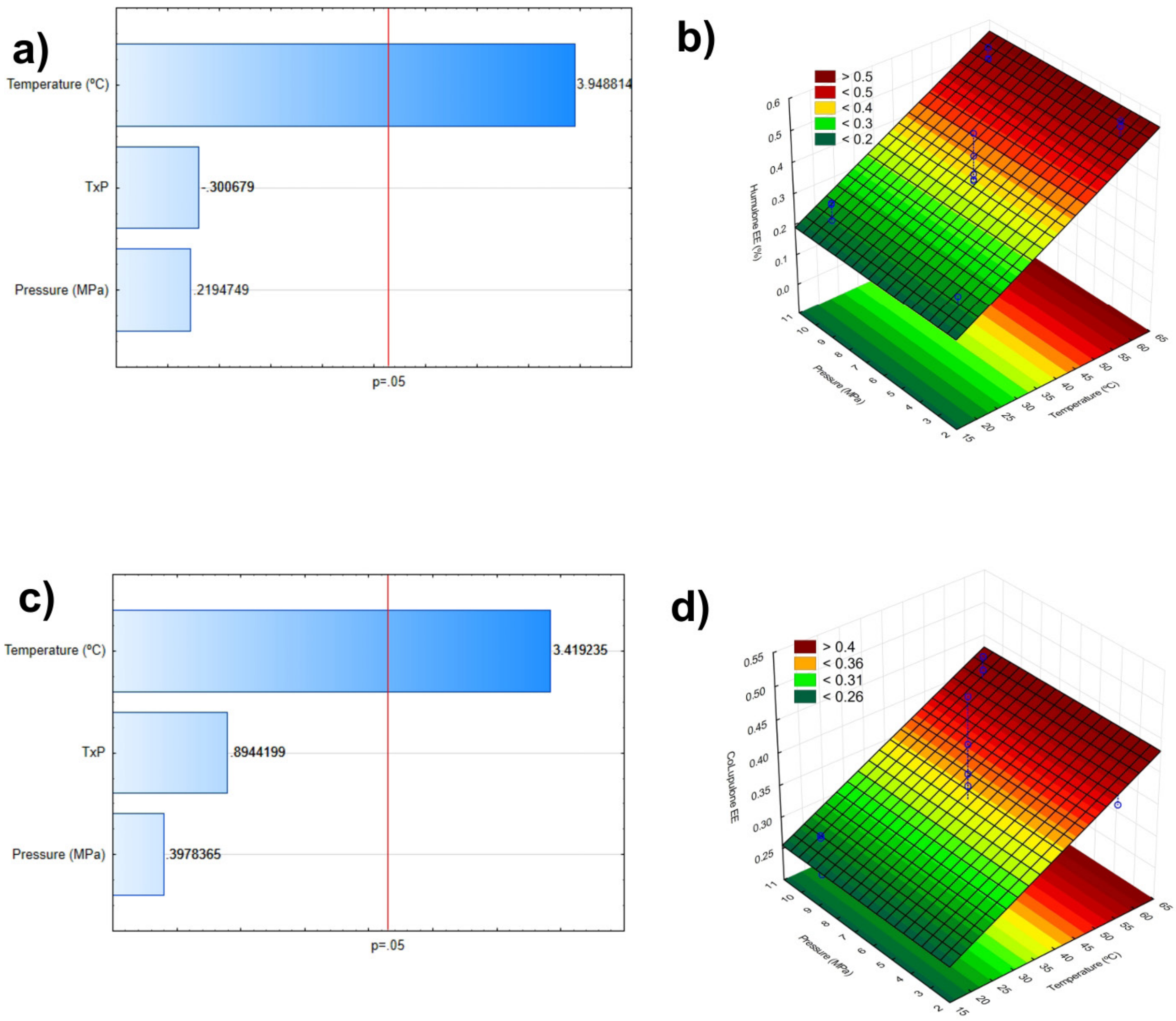
Despite its effectiveness in extracting some compounds, pressurised propane was ineffecient in the extraction of xanthohumol. This is likely to be due to the nonpolar nature of propane, which results in weak interactions with the polar flavonoids in hops, such as xanthohumol (Klimek et al. 2021). Conversely, propane demonstrated a high affinity for hydrophobic acids, making it a promising solvent for obtaining extracts rich in alpha and beta acids.

Temperature was the main factor affecting the extraction efficiencies (EE) of alpha and beta acids. As shown in the Pareto diagrams (Figures 3a and 3b), the impact of temperature outweighed any pressure related effect. The increase in temperature enhanced the solvation power of propane, improving the extraction efficiencies of these compounds. This behaviour aligns with the principles of subcritical fluid extraction, where elevated temperatures promote diffusion and solubility while also accelerating mass transfer (Bizaj et al. 2022).

Response surface analysis was conducted to model the extraction efficiencies of humulone and lupulone, the most abundant alpha and beta acids in the extracts. The response surfaces (Figures 3c and 3d) reflect the significant role of temperature in improving the yields of these compounds, while pressure showed a negligible effect across the range. The mathematical models derived for these response surfaces confirm that temperature is the primary driver of extraction efficiency with propane.

Figure 3.

Pareto charts (a, c) and response surface plots (b, d) showing the effects of process parameters on the extraction efficiency (EE) of humulone and colupulone from Mantiqueira hops using pressurised propane. Extraction efficiency equations: EE (%) = 7.801+0.7476·*T* for humulone, and EE (%) = 20.25+0.352·*T* for colupulone.



The selectivity of pressurised propane for alpha and beta acids over polyphenols offers practical advantages. The reduced extraction of polyphenols minimises astringency, enhancing the palatability of the resulting extracts. This characteristic makes propane extraction of resins particularly suitable for addition during wort boiling in beer production, where they would contribute a clean, smooth bitterness without excessive astringency.

Co-solvent extraction with scCO$_2$

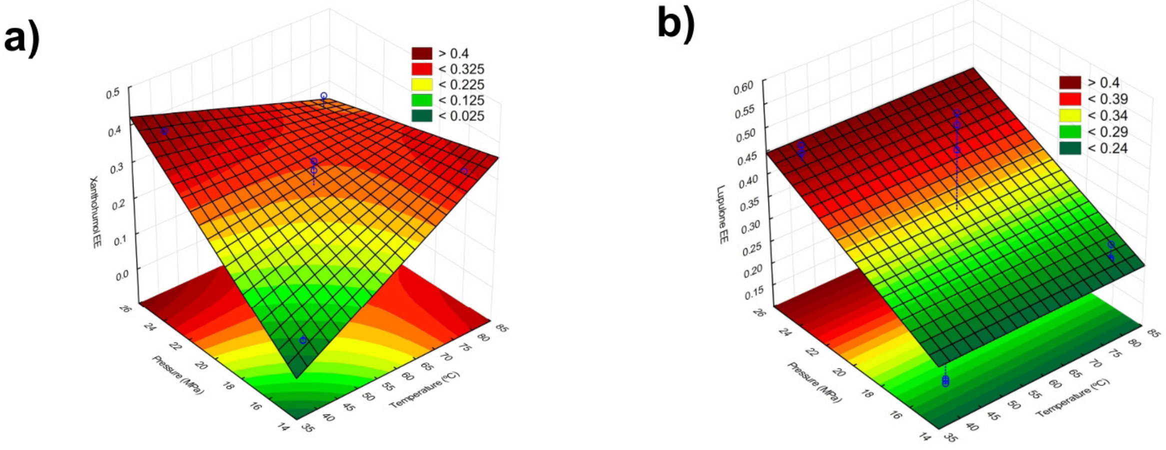
Extractions using ethyl acetate (EtOAc) as an organic co-solvent with scCO$_2$ were analysed at two different mass ratios (MRs) between the co-solvent and the initial mass of hops (runs 15-28, Table 1). The extraction yield with scCO$_2$ alone was lower than obtained with other techniques. The introduction of an organic co-solvent mitigates this limitation by enhancing the affinity between the solvent mixture and the phenolic compounds of the plant matrix. The results are presented based on the solvent-to-matrix (MR): one set of assays with an MR of 1, while the other an MR of 2.

Extraction with scCO$_2$ + ethyl acetate at an MR = 1

The overall extraction yield was mainly dependent on the pressure. Higher extraction pressures led to increased extract mass per gram of the hop matrix. The addition of ethyl acetate (MR = 1) slightly

Figure 4.

Response surface plots showing the effects of process parameters on the extraction efficiency (EE) of xanthohumol (a) and lupulone (b) from Mantiqueira hops using supercritical CO_2 + ethyl acetate at MR 1 as co-solvent. Extraction efficiency equations: EE (%) = $-11.23+1.895 \cdot T+6.404 \cdot P-0.0862 \cdot TP$ for xanthohumol, and EE (%) = $-1.159+2.210 \cdot P$.



improved the overall yield and altered the extract composition. The use of a co-solvent increased the effeciency of xanthohumol extraction by over 30-fold, raising its molar fraction from <1 to 5.1%. This enhancement was accompanied by increased efficiency of alpha acid extraction with a slight reduction in beta acids. The reduction in lupulone concentration may favour the use of these extracts during maturation and packaging of beer. Studies have shown that the use of ethyl acetate as a co-solvent in supercritical CO_2 extractions improves the efficiency of target compound extraction. This was effective in enhancing the extraction of caffeine from green tea (Bermejo et al. 2016) and triterpenoids from *Acacia dealbata* leaves (Rodrigues et al. 2023).

Statistical analysis indicated that temperature, pressure, and their interactions significantly influenced the effeciency of xanthohumol efficiency. Response surface models, using pressure and temperature as variables, were constructed to describe this behaviour (Figure 4a). The findings highlight that continuous extraction in a fixed bed with a co-solvent under high pressure is the most effective way to extract xanthohumol. Future studies expanding the pressure range could further optimise this process, which offers potential for producing bioactive extracts.

While the extraction efficiencies of alpha acids had limited sensitivity to temperature and pressure, the beta acids (lupulone and colupulone) showed greater sensitivity, with their extraction efficiencies directly proportional to increases in pressure. A response surface plot illustrating lupulone's extraction efficiency as a function of temperature and pressure is reported in Figure 4b.

Extraction with $scCO_2$ + ethyl acetate at an MR = 2

Increasing the co-solvent MR to 2 significantly enhanced the overall extraction yield. Both temperature and pressure positively influenced the yield, with a combination of these variables having the most pronounced effect. As expected, this configuration yielded the highest efficiency of xanthohumol extraction and molar fraction, confirming the trend observed with an MR of 1. The increased proportion of co-solvent enhanced the extraction of phenolic compounds while achieving the highest efficiency of alpha acid extraction in this work. However, the efficiency of beta acid extraction showed a stronger dependence on extraction conditions (Kupski et al. 2017; Klimek et al. 2021).

Temperature and pressure played distinct roles in the extraction process, with the effects varying across different compounds. Although counterintuitive pressure had a negative effect on the effeciency of extraction of alpha and beta compounds, despite its positive influence on the overall yield.

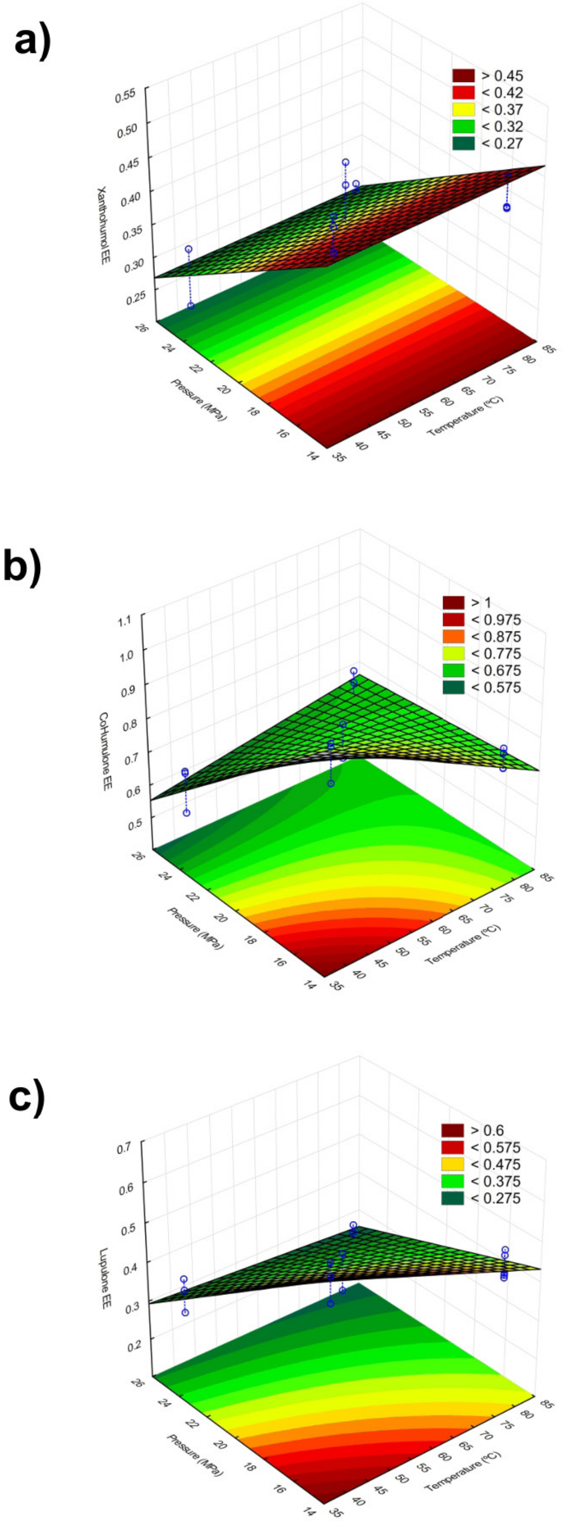
This discrepancy may arise from the increased solvent density at higher pressures, which reduce the ability of the solvent-co-solvent mixture to selectively carry denser compounds (Bermejo et al. 2015; Pinto et al. 2023). While the overall yield increased, the extract composition became more diverse, diluting the targeted compounds.

A similar trend was seen with temperature and the efficiency of extraction of alpha and beta compound. At higher temperatures, the increased solubility of lower molecular weight compounds and enhanced interactions with polar molecules favoured their extraction over alpha/beta acids. A thorough examination of this system would help identify the best conditions for obtaining (potential) bitter, phenolic, and flavour compounds. Nonetheless, the use of ethyl acetate resulted in extracts rich in alpha/beta and xanthohumol compounds while increasing the overall extraction yield.

Response surfaces were constructed to illustrate the behaviour of the efficiencies of extraction for xanthohumol, alpha acids (represented by cohumulone), and beta acids (represented by lupulone) as a function of temperature and pressure (Figures 5a–5c). Figure 5a shows that increasing pressure reduced the amount of xanthohumol extracted per initial hop mass in the fixed bed. For alpha acids, both pressure and temperature negatively affected extraction, with the lowest temperature and pressure yielding the highest efficiency, conditions where the co-solvent effect is predominant in relation to $scCO_2$ (Figure 5b). As the mixture appears in a subcritical state at higher temperature and pressure, the viscosity of the mobile phase tends to be higher, hindering the mass transfer by the formation of a film-type interface between the plant matrix and the solvent-co-solvent mixture. Similar behaviour was observed for beta acids (Figure 5c).

Figure 5.

Response surface plots showing the effects of process parameters on the extraction efficiency (EE) of (a) xanthohumol, (b) cohumulone, and (c) lupulone from Mantiqueira hops using supercritical CO_2 + ethyl acetate at MR 2 as co-solvent. Extraction efficiency equations: EE (%) = $69.55-1.641\cdot P$ for xanthohumol; EE (%) = $223.7-1.745\cdot T-6.760\cdot P+0.0753\cdot TP$ for cohumulone; and EE (%) = $197.4-1.274\cdot T-5.752\cdot P+0.0424\cdot TP$ for lupulone.



Extraction with $scCO_2$ + ethanol

The use of ethanol as a co-solvent with supercritical carbon dioxide (runs 29-31) enhanced the extraction of polar compounds from Brazilian Mantiqueira hops. Table 1 shows that the extraction yield increases with temperature. Ethanol, being a polar solvent, improved the solubility and recovery of bioactive compounds such as xanthohumol and phenolic compounds, which are less soluble in $scCO_2$.

The concentration of compounds varied across $scCO_2$ + ethanol extractions (Table 2). Besides providing the highest yield, the extract obtained at 80°C and 25 MPa showed the highest levels of humulone, cohumulone, and xanthohumol. Conversely, at 60°C and 20 MPa, a marked increase in xanthohumol concentration was observed, but with lower extraction efficiency for humulone and cohumulone. This suggests a preference for the extraction of bioactive compounds like xanthohumol at the higher temperature. These values reflect the ability of the $scCO_2$ + ethanol system to efficiently extract both alpha acids and bioactive compounds.

These findings suggest that $scCO_2$ combined with ethanol is a promising method for extraction of both alpha/beta compounds and bioactive molecules, with higher temperature and pressure generally yielding better results in terms of both extraction efficiency and compound diversity. However, the specific composition of the extract varies depending on the extraction conditions, highlighting the need for optimisation based on the desired outcome, such bioactive compounds (xanthohumol) or compounds like humulone.

Conclusions

This study explores the extraction of the most abundant compounds in Brazilian Mantiqueira hops, with guidance in developing new approaches involving $scCO_2$ and co-solvents under subcritical and supercritical conditions. Using HPLC, seven key compounds were identified and quantified: three alpha acids (humulone, cohumulone, and adhumulone), three beta acids (lupulone, colupulone, and adlupulone), and xanthohumol. The extracts obtained solely with $scCO_2$ showed remarkable selectivity, representing 93.6–98.9% of the total mass. In contrast, extracts from pressurised propane and $scCO_2$ + cosolvents showed varying selectivity, with the quantified compounds representing respectively 54.4–80.8% and 53.0–99.0%. The results highlight the adaptability of $scCO_2$ as a selective solvent and the potential of ethyl acetate as a co-solvent to enhance the efficiency of exytraction. The composition of Brazilian Mantiqueira hops was comparable to noble hops of the Czech Republic and the aroma rich North American hops. This suggests a potential role for these hops in the production of beer with desirable sensory characteristics. Furthermore, the level of xanthohumol underscores the potential of Brazilian hops for producing bioactive extracts with antioxidant, anti-inflammatory, and anticancer properties.

Each extraction methodology showed promise for specific applications. Pressurised propane was most effective for extracting alpha/beta acids while minimising phenolic content, which is ideal for applications requiring minimal astringency. $scCO_2$ showed versatility in extracting efficiently both alpha/beta acids and xanthohumol. The $scCO_2$-ethyl acetate mixture was highly efficient for alpha acids and xanthohumol, making it suitable for producing beer with clean bitterness profiles and reduced astringency.

Overall, this study demonstrates the potential of Brazilian hops as a high value raw material for beverage and functional food applications. Further research should focus on (i) optimising extraction parameters, such as temperature, pressure, and co-solvent ratios and (ii) to tailor the process for specific industrial applications, while combining sensory studies with process optimisation to leverage the potential of flavour and bioactive compounds.

Acknowledgements

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

Conflict of interest

The authors declare there are no conflicts of interest.

Author contributions

Bruno A Veiga: Conceptualisation, investigation, data curation and writing (original draft).
Maria C Roda-Serrat: Methodology, investigation, and writing (review).
Mathias P Clausen: Conceptualisation and investigation.
Massimiliano Errico: Data curation, writing (review) and supervision.
Marcos L Corazza: Data curation, writing (review) and funding acquisition.
Agnes P Scheer: Conceptualisation, investigation, data curation, writing (review and editing), funding acquisition and project administration.

References

Andrade TA. 2018. *Energy-efficient conversion of castor oil for biodiesel production: Investigate novel energy-efficient biodiesel production technologies such as enzymes and membranes.* University of Southern Denmark, Faculty of Engineering. https://portal.findresearcher.sdu.dk/en/publications/energy-efficient-conversion-of-castor-oil-for-biodiesel-productio

Arruda TR, Pinheiro PF, Silva PI, Bernardes PC. 2022. Exclusive raw material for beer production? Addressing greener extraction techniques, the relevance, and prospects of hops (*Humulus lupulus* L.) for the food industry. *Food Bioproc Tech* 15:275–305. https://doi.org/10.1007/s11947-021-02716-w

Bermejo DV, Ibáñez E, Reglero G, Fornari T. 2016. Effect of cosolvents (ethyl lactate, ethyl acetate and ethanol) on the supercritical CO_2 extraction of caffeine from green tea. *J Supercrit Fluids* 107:507–512. https://doi.org/10.1016/j.supflu.2015.07.008

Bermejo DV, Mendiola JA, Ibáñez E, Reglero G, Fornari T. 2015. Pressurized liquid extraction of caffeine and catechins from green tea leaves using ethyl lactate, water and ethyl lactate + water mixtures. *Food Bioprod Process* 96:106–112. https://doi.org/10.1016/j.fbp.2015.07.008

Bessada SMF, Barreira JCM, Barros L, Ferreira ICFR, Oliveira MBPP. 2016. Phenolic profile and antioxidant activity of *Coleostephus myconis* (L.) Rchb.f.: An underexploited and highly disseminated species. *Ind Crops Prod* 89:45–51. https://doi.org/10.1016/j.indcrop.2016.04.065

Bizaj K, Škerget M, Košir IJ, Knez Ž. 2021. Sub- and supercritical extraction of Slovenian hops (*Humulus lupulus* L.) Aurora variety using different solvents. *Plants* 10:1137. https://doi.org/10.3390/plants10061137

Bizaj K, Škerget M, Košir IJ, Knez Ž. 2022. Hop (*Humulus lupulus* L.) Essential oils and xanthohumol derived from extraction process using solvents of different polarity. *Horticulturae* 8:368. https://doi.org/10.3390/horticulturae8050368

Bocquet L, Sahpaz S, Hilbert JL, Rambaud C, Rivière C. 2018. *Humulus lupulus* L. a very popular beer ingredient and medicinal plant: overview of its phytochemistry, its bioactivity, and its biotechnology. *Phytochem Rev* 17:1047–1090. https://doi.org/10.1007/s11101-018-9584-y

Bolton JL, Dunlap TL, Hajirahimkhan A, Mbachu O, Chen S-N, Chadwick L, Nikolic D, van Breemen RB, Pauli GF, Dietz BM. 2019. The multiple biological targets of hops and bioactive compounds. *Chem Res Toxicol* 32:222–233. https://doi.org/10.1021/acs.chemrestox.8b00345

Dias GS, Gallon ME, Gobbo-Neto L. 2024. Comparative analysis of four hop cultivars grown in Brazil and the USA by GC-MS-based metabolomics. *J Inst Brew* 130:238-249. https://doi.org/10.58430/jib.v130i4.62

Dresel M, Vogt C, Dunkel A, Hofmann T. 2016. The bitter chemodiversity of hops (*Humulus lupulus* L.). *J Agric Food Chem* 64:7789–7799. https://doi.org/10.1021/acs.jafc.6b03933

Duarte PF, Nascimento LH, Fischer B, Lohmann AM, Bandiera VJ, Fernandes IA, Magro JD, Valduga E, Cansian RL, Paroul N, Junges A. 2023. Effect of extraction time on the yield, chemical composition, and antibacterial activity of hop essential oil against lactic acid bacteria (*Lactobacillus brevis* and *Lactobacillus casei*) beer spoilage. *Curr Microbiol* 80:237. https://doi.org/10.1007/s00284-023-03359-0

Goiris K, Jaskula-Goiris B, Syryn E, Van Opstaele F, De Rouck G, Aerts G, De Cooman L. 2014. The flavoring potential of hop polyphenols in beer. *J Am Soc Brew Chem* 72:135–142. https://doi.org/10.1094/ASBCJ-2014-0327-01

Herkenhoff ME, Brödel O, Frohme M. 2024. Hops across continents: exploring how terroir transforms the aromatic profiles of five hop (*Humulus lupulus*) varieties grown in their countries of origin and in Brazil. *Plants* 13:2675. https://doi.org/10.3390/plants13192675

Jastrombek JM, Faguerazzi MM, Pierezan HC, Rufato L, Sato AJ, Ricce WS, Marques VV, Leles NR, Roberto SR. 2022. Hop: an emerging crop in subtropical areas in Brazil. *Horticulturae* 8:393. https://doi.org/10.3390/horticulturae8050393

Karabín M, Hudcová T, Jelínek L, Dostálek P. 2016. Biologically active compounds from hops and prospects for their use. *Compr Rev Food Sci Food Saf* 15:542–567. https://doi.org/10.1111/1541-4337.12201

Klimek K, Tyśkiewicz K, Miazga-Karska M, Dębczak A, Rój E, Ginalska G. 2021. Bioactive compounds obtained from Polish "Marynka" hop variety using efficient two-step supercritical fluid extraction and comparison of their antibacterial, cytotoxic, and anti-proliferative activities in vitro. *Molecules* 26:2366. https://doi.org/10.3390/molecules26082366

Kljakić AC, Ocvirk M, Rutnik K, Košir IJ, Pavlić B, Mašković P, Mašković J, Teslić N, Stupar A, Uba AI, Zengin G. 2024. Exploring the composition and potential uses of four hops varieties through different extraction techniques. *Food Chem* 447:138910. https://doi.org/10.1016/j.foodchem.2024.138910

Kupski SC, Klein EJ, Silva EA, Palú F, Guirardello R, Vieira MGA. 2017. Mathematical modeling of supercritical CO2 extraction of hops (*Humulus lupulus* L.). *J Supercrit Fluids* 130:347–356. https://doi.org/10.1016/j.supflu.2017.06.011

Liu M, Hansen P, Wang G, Qiu L, Dong J, Yin H, Qian Z, Yang M, Miao J. 2015. Pharmacological profile of xanthohumol, a prenylated flavonoid from hops (*Humulus lupulus*). *Molecules* 20:754–779. https://doi.org/10.3390/molecules20010754

Lupinacci E, Meijerink J, Vincken J-P, Gabriele B, Gruppen H, Witkamp RF. 2009. Xanthohumol from hop (*Humulus lupulus* L.) is an efficient inhibitor of monocyte chemoattractant protein-1 and tumor necrosis factor-α release in LPS-stimulated RAW 264.7 mouse macrophages and U937 human monocytes. *J Agric Food Chem* 57:7274–7281. https://doi.org/10.1021/jf901244k

Machado JC, Faria MA, Ferreira IMPLVO, Páscoa RNMJ, Lopes JA. 2018. Varietal discrimination of hop pellets by near and mid infrared spectroscopy. *Talanta* 180:69–75. https://doi.org/10.1016/j.talanta.2017.12.030

Metaj I, Hajdini D, Gliha K, Košir IJ, Ocvirk M, Kolar M, Cerar J. 2023. Extraction of polyphenols from Slovenian hop (*Humulus lupulus* L.) Aurora variety using deep eutectic solvents: choice of the extraction method vs. structure of the solvent. *Plants* 12:2890. https://doi.org/10.3390/plants12162890

Overk CR, Guo J, Chadwick LR, Lantvit DD, Minassi A, Appendino G, Chen S-N, Lankin DC, Farnsworth NR, Pauli GF, van Breemen RB, Bolton JL. 2008. In vivo estrogenic comparisons of *Trifolium pratense* (red clover) *Humulus lupulus* (hops), and the pure compounds isoxanthohumol and 8-prenylnaringenin. *Chem Biol Interact* 176:30–39. https://doi.org/10.1016/j.cbi.2008.06.005

Paniagua-García AI, Ruano-Rosa D, Díez-Antolínez R. 2023. Fractionation of high-value compounds from hops using an optimised sequential extraction procedure. *Antioxidants* 13:45. https://doi.org/10.3390/antiox13010045

Pinto MBC, Vardanega R, Náthia-Neves G, França PRL, Kurozawa LE, Meireles MAA, Schmidt FL. 2023. Novel Brazilian hop (*Humulus lupulus* L.) extracts through supercritical CO_2 extraction: Enhancing hop processing for greater sustainability. *Food Res Int* 172:113169. https://doi.org/10.1016/j.foodres.2023.113169

Rodrigues VH, Portugal I, Silva CM. 2023. Experimental optimization of the supercritical fluid extraction of triterpenoids from *Acacia dealbata* Link. leaves. *Sep Purif Technol* 306:122637. https://doi.org/10.1016/j.seppur.2022.122637

Rój E, Tadić VM, Mišić D, Žižović I, Arsić I, Dobrzyńska-Inger A, Kostrzewa D. 2015. Supercritical carbon dioxide hops extracts with antimicrobial properties. *Open Chem* 13:1157–1171. https://doi.org/10.1515/chem-2015-0131

Sanz V, Torres MD, Vilarino JML, Dominguez H. 2022. Green extraction of phenolic compounds from Perle Hallertau and Nuggets hop pellets. *Food Biosci* 50:102044. https://doi.org/10.1016/j.fbio.2022.102044

Teghtmeyer S. 2018. Hops. *J Agric Food Inf* 19:9–20. https://doi.org/10.1080/10496505.2018.1403248

Veiga BA, Hamerski F, Clausen MP, Errico M, Scheer AP, Corazza ML. 2021. Compressed fluids extraction methods, yields, antioxidant activities, total phenolics and flavonoids content for Brazilian Mantiqueira hops. *J Supercrit Fluids* 170:105155. https://doi.org/10.1016/j.supflu.2020.105155

Zekovic Z, Pfaf-Sovljanski I, Grujic O. 2007. Supercritical fluid extraction of hops. *J Serb Chem Soc* 72:81–87. https://doi.org/10.2298/JSC0701081Z