Date: 01.01.2025

Analysis report – SFE and SWE extracts

Description of sample(s)

Sample name: Extracts of hops

Sample type: Supercritical CO_2 (SFE) and subcritical water (SWE) extracts

Scientific name: *Humulus lupulus*

Manufacturing date: -

Volume/mass of sample(s): A total of 189 samples were taken for analysis (10.0–30.0 ± 0.1 mg)

Customer: -

Applied methods of sample preparation and analysis

Analysis date: 21.11.2024 – 22.12.2024

Analyst: Dr. Deniz Can Köseoğlu

Sample preparation:

Bitter α- and β-acids, iso-acids, xanthohumol: Dilution in pure methanol at a concentration of 0.3–3.0 ± 0.1 mg mL^{-1}

Analyses and additional services:

Test supercritical fluid extractions (SFE using CO_2) and determination of extract yield (%)

Construction of the Overall Extraction Curve (OEC) from SFE data

Gravimetric analysis of moisture content (%)

Isolation of α- and β-acids from whole SFE extract into an aqueous alkaline solution (as potassium salts)

Isomerization of alpha-acids (as potassium salts) into iso-alpha-acids using heat

Extraction and purification of xanthohumol from spent hops (residues left after SFE extraction)

HPLC-DAD analysis of bitter α- and β-acids (cohumulone, *n*-/adhumulone, colupulone, *n*-/adlupulone)

HPLC-DAD analysis of iso-alpha-acids and xanthohumol

Checked and approved by:

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Supercritical CO_2 extraction (SFE)

Sample preparation of hops consisted of grinding of type 90 pellets on a cutter with a blade speed of up to 28000 rpm to a particle diameter of ≤1 mm. The moisture content of all samples was ≤10% for SFE compatibility.

SFE extractions were carried out on a SuperEx SC-1000 system with an extraction vessel of 1 L able to withstand maximum operating pressure and temperature of 500 bar and 200 °C, respectively. The raw material was placed in the vessel using a steel loading basket equipped with 10 µm mesh filters. After sealing, the vessel was heated to the set temperature and pressurized. Upon reaching the specified temperature and pressure, extraction was carried out in a dynamic (flow-through) mode with a constant flow of CO_2. The outflow pressure after exiting the vessel was released by an automatic backpressure regulator, settling the extract in the separator. After the specified extraction time or the required solvent to raw material ratio was reached, the extract was collected using a built-in needle valve.

A total of 5 varieties of hops were extracted, including Brewer's Gold (**BG**), Aroma (**AR**), Magnum (**MG**), Spalter Selekt (**SP**), and Traditional (**TD**). The objective of SFE was extraction of bitter α- and β-acids (i.e. humulones and lupulones) with and without fractionation. The α-acids evalauted included cohumulone (hereafter **CoH**), *n*-humulone, and adhumulone (collectively referred to as ***n*-/AdH**), while β-acids included colulupone (**CoL**) as well as both *n*- and adlupulone (***n*-/AdL**). For **BG** and **AR** varieties, pure CO_2 at 300 or 450 bar was circulated through hops at a flow rate of 100 mL min^{-1} and a temperature of 40 °C for 2.5 hours. In both cases, fractionation of the less soluble α-acids was attempted by setting the first separator to 150 bar. A separate extraction without fractionation was also carried out at 450 bar. SFE of **MG**, **SP**, and **TD** hops was carried out at 450 bar. For **MG** only, the ability of SFE to actively remove cuticular waxes from the extract was also tested separately by cooling the first separator to -10 °C at this pressure. Finally, to evaluate the extraction rate and progression of bitter acid recovery, **MG** hops were extracted at 150 bar (60 min) followed by 300 bar (120 min) with periodic extract collection and analysis. Three parameters were analysed in all extracts: the overall extraction yield (% of loaded material), concentration of bitter acids (% extract), and their recovery from the raw material (mg g^{-1} d.w.). All extractions and their parameters are summarized in the table below.

Ext.	Extraction Conditions					Material loading (g)				
#	*P* (bar)	*T* (°C)	Time (min)	*P* in S1* (bar)	*T* in S1 (°C)	BG	MG	AR	SP	TD
1	300	40	150	150	40	259.68	✕	266.82	✕	✕
2	450	40	150	150	40	260.39	249.10	247.82	199.98	179.21
3	450	40	150	55	40	120.66	✕	121.59	✕	✕
4	450	40	150	150	**-10**	✕	224.24	✕	✕	✕
5	150	40	60	55	40	✕	200.12	✕	✕	✕
	300	40	120	55	40					

*Separator 1

Subcritical water (SWE) extraction of spent hops

Xanthohumol extraction of spent hops with subcritical water was attempted in both dynamic (continuous flow of 30 mL min^{-1}) and static mode using the SC-1000 system. Thus, a total of two extractions were carried out at 150 °C for a period of 20 minutes. The extract solutions were then lyophilized for 30 hours at -40 °C.

Separation of alpha- and beta-acids from whole extracts into separate fractions

Whole or SFE-fractionated extracts were heated to 60 °C to decrease their viscosity and ca. 100 g were slowly added to a round-bottom flask (1000 mL) containing 385 mL of aqueous KOH (0.5 M) under stirring, such that the final concentration of alpha-acids in this initial solution was ca. 15% (*w/w*) at a mole ratio of divalent

alpha-acids to monovalent KOH of ca. 1.2. An analogous method with a 1.92 mol equivalent and 1.78 M KOH was also used for comparative purposes. The resulting solution was refluxed in a Clevenger apparatus for 45 min (100 °C) to convert alpha-acids to their potassium salts and simultaneously distil essential oil from extracts. The supernatant aqueous layer was then separated by centrifugation (5000 rpm, 5 min), filtered under vacuum, and concentrated to <100 mL on a rotary evaporator. The remaining solid residue containing beta-acids was refluxed with a further 385 mL of KOH solution, with the supernatant retrieved and concentrated similarly to the previous step.

Isomerization of alpha-acids

Concentrated potassium salts of alpha-acids were isomerized by heating to 100 °C for approximately 6 hours. High pressure flash isomerization was not attempted at this stage.

Extraction and purification of xanthohumol from spent hops after SFE

Approximately 100 g of spent hops (residues after SFE) were accurately weighed and extracted thrice with 400 mL of pure methanol under stirring at 40 °C. The reaction mixture was filtered under vacuum and the residue washed with additional methanol. The filtrate was concentrated to ca. 80 mL using a rotary evaporator and the concentrated solution was well-mixed with ca. 100 g of kieselguhr (diatomaceous earth) before drying at 60 °C for 2 hours. The kieselguhr was loaded into a glass chromatography column and compacted under vacuum. Two fractions were eluted successively with 400 mL of 30% MeOH : water (*v/v*) and 500 mL of 60% MeOH : water (*v/v*). Xanthohumol was precipitated from the latter fraction by adding 100 mL of distilled water under stirring. Following vacuum filtration and washing with distilled water, the precipitate was dried at 60 °C for 2 hours.

HPLC-DAD analysis of bitter acids, isomerised alpha-acids, and xanthohumol in extracts

A sample of the extract (10.0–30.0 ± 0.1 mg) was diluted in pure methanol (MeOH) at a concentration of 1.0–3.0 ± 0.1 mg ml^{-1} in a glass vial, shaken by hand (1 min) and vortexed (1 min) until complete dissolution. An aliquot of 1 ml was passed through a membrane filter (PVDF, 0.22 µm) into a vial for HPLC-DAD analysis (injection volume 10 µl). The International Calibration Extract 4 (ICE-4) standard solution was prepared in triplicate via the same method.

A Shimadzu i-Series LC-2050-3D liquid chromatograph with diode array detector and a C_{18} column (250 × 4.6 mm, 5 µm) were used. Isocratic elution (27 min) was carried out with pure MeOH and 775:215:10 MeOH:H_2O:H_3PO_4 as mobile phases A and B at a composition of 35 : 65 (*v/v*) and a flow rate of 1.0 mL min^{-1} and a column temperature set to 40 °C. Peak area measurements were performed at 314 nm.

A slightly different HPLC method was used for analysis of iso-alpha-acids and xanthohumol. The same mobile phases were used with the following gradient: 100% B (held for 17 min) to 65% B (held for 13 min) and back to 100% B (held for 5 min). Iso-acids and xanthohumol were detected at 270 nm and 370 nm, respectively.

GC-MS and GC-FID analysis of hops essential oils

Essential oils were diluted 100-fold in hexane before GC analysis. Hydrogen was used as the carrier gas. Injection was carried out in split mode (split ratio 50:1) at 200 °C using an HP5-MS column (30 m × 0.25 mm × 0.25 µm). The transfer line (or FID detector) was kept at 260 °C, and the ion source at 230 °C. The column temperature was programmed as follows: 50 °C raised to 160 °C at 3 °C min^{-1}, then to 260 °C at 20 °C min^{-1} (held for 5 min). The total runtime was 48 min.

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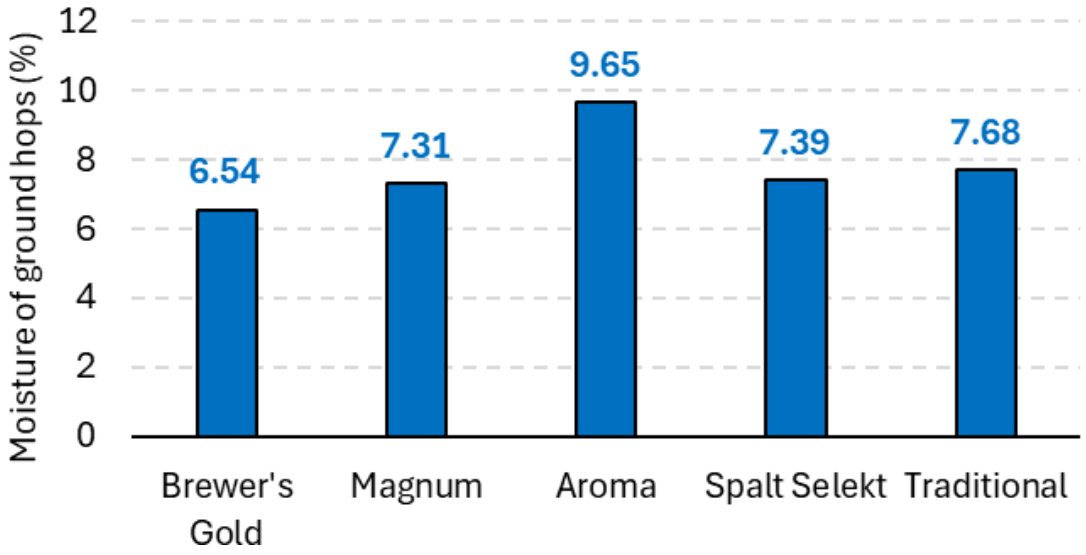
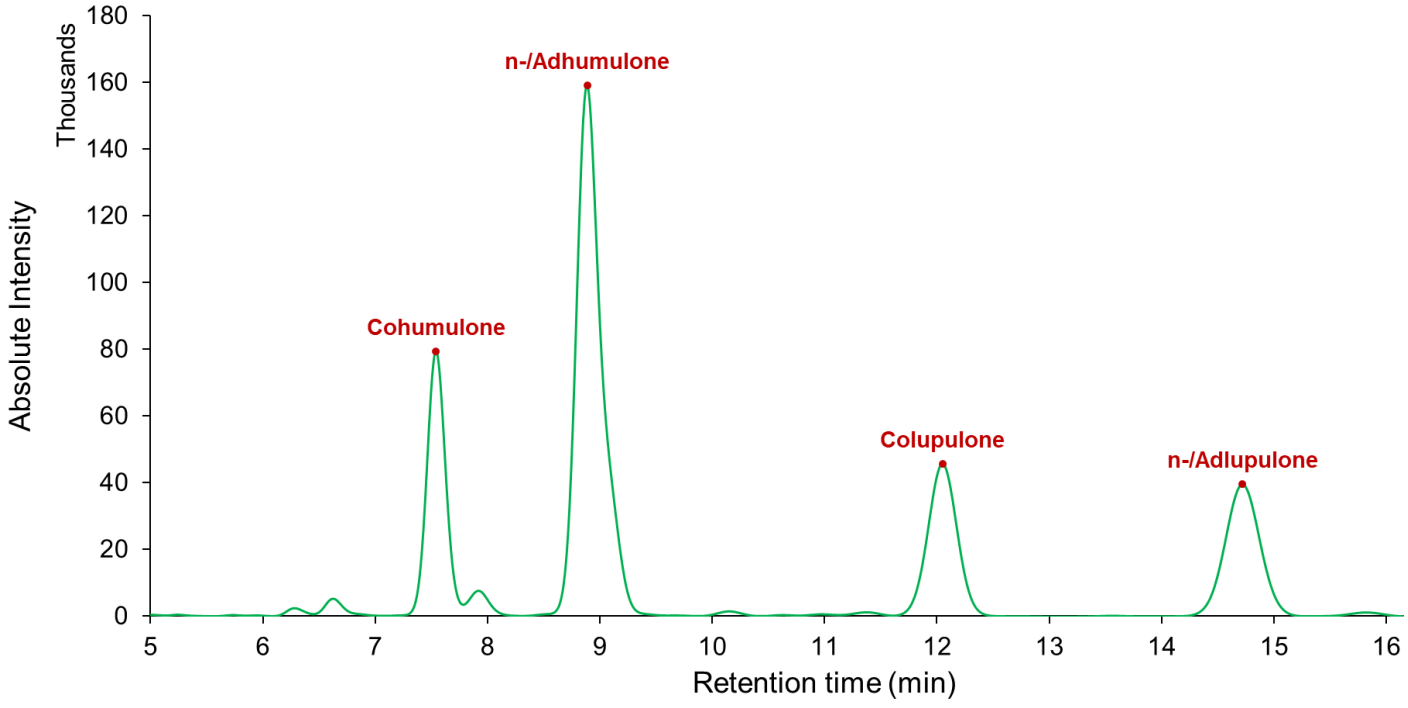
General information – Moisture content in comminuted hop samples

Hop variety	Measurements (%)			Mean ± SD
	1	2	3	
Brewer's Gold (**BG**)	6.45	6.56	6.62	6.54 ± 0.09
Aroma (**AR**)	7.23	7.14	7.56	7.31 ± 0.22
Magnum (**MG**)	9.73	9.59	9.62	9.65 ± 0.07
Spalter Selekt (**SP**)	7.62	7.13	7.43	7.39 ± 0.25
Traditional (**TD**)	8.02	7.43	7.59	7.68 ± 0.31



General Information – Example chromatogram of bitter acids (HPLC-DAD)



Extracts of hops *(Humulus lupulus)*, supercritical CO_2 (SFE) and subcritical water (SWE)
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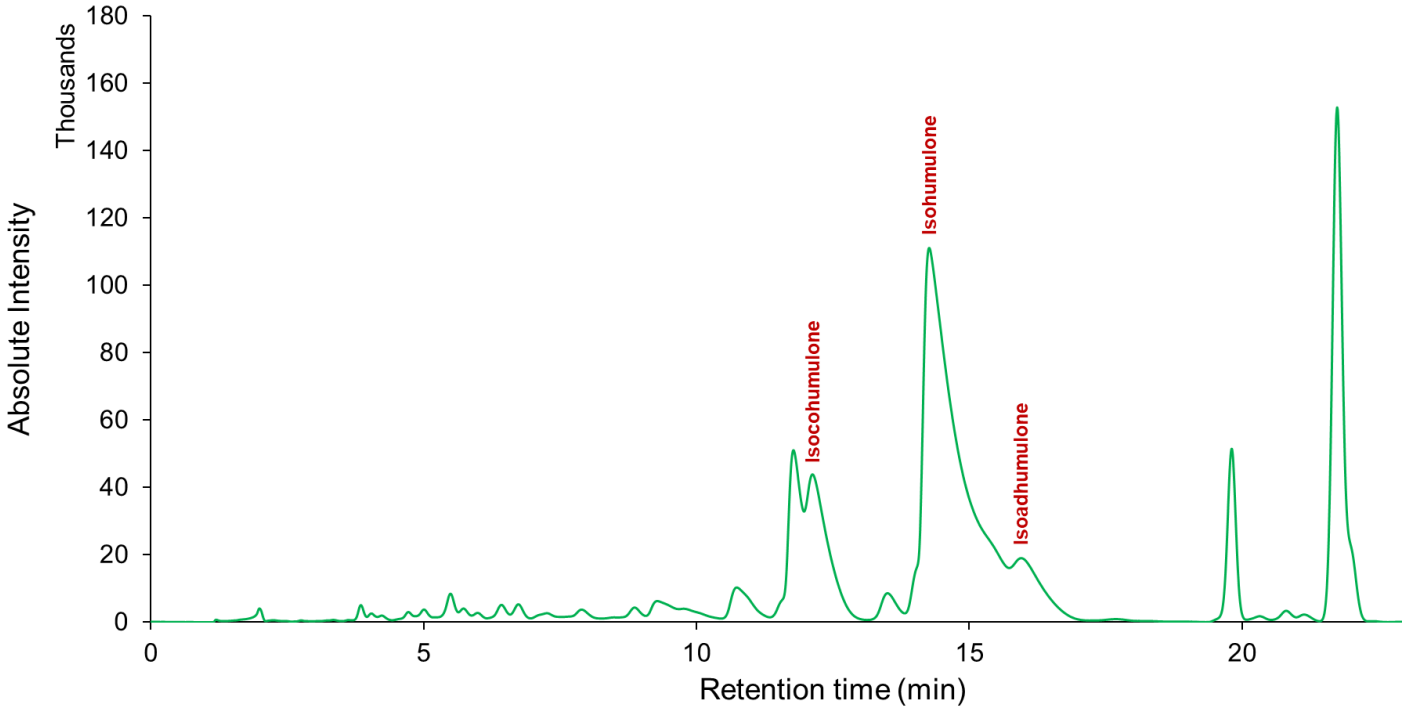
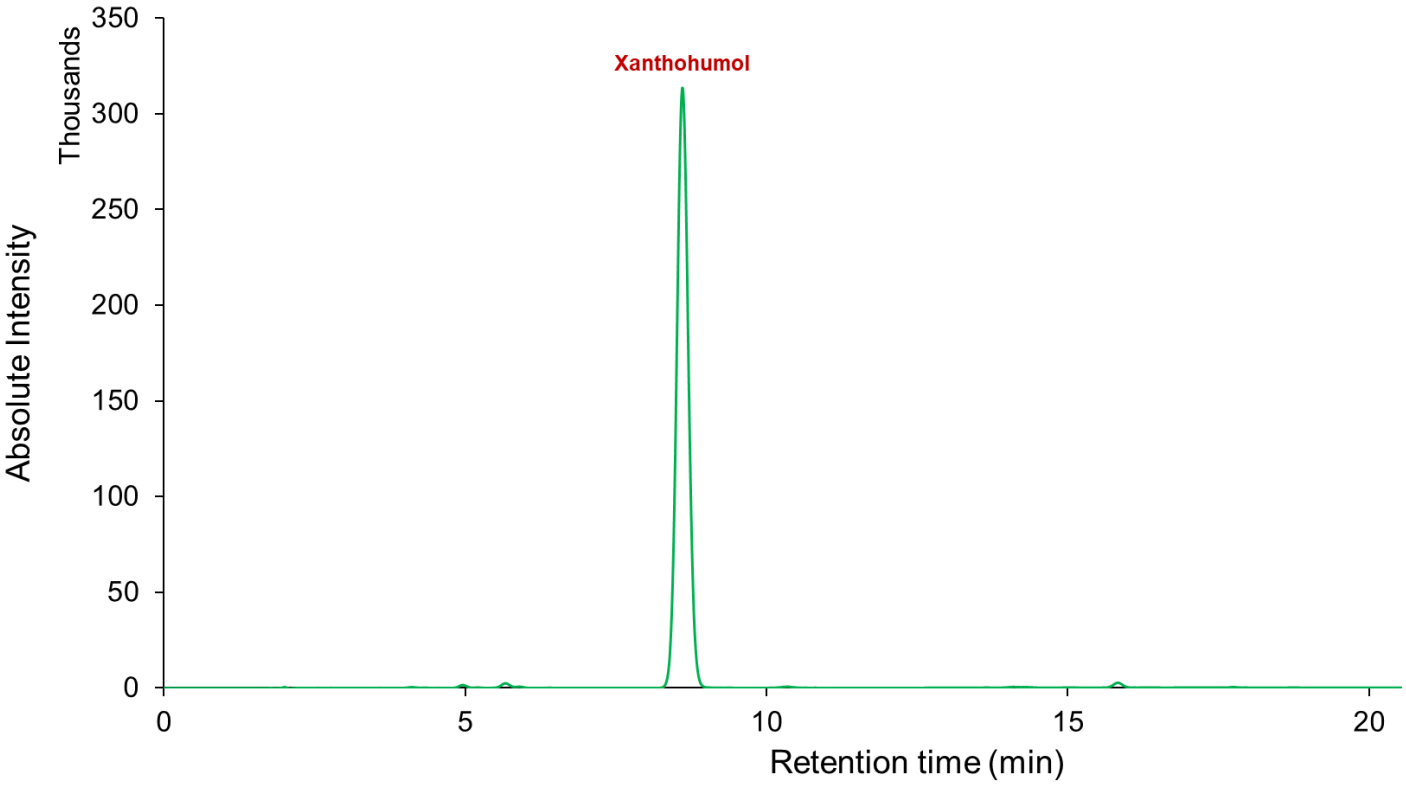
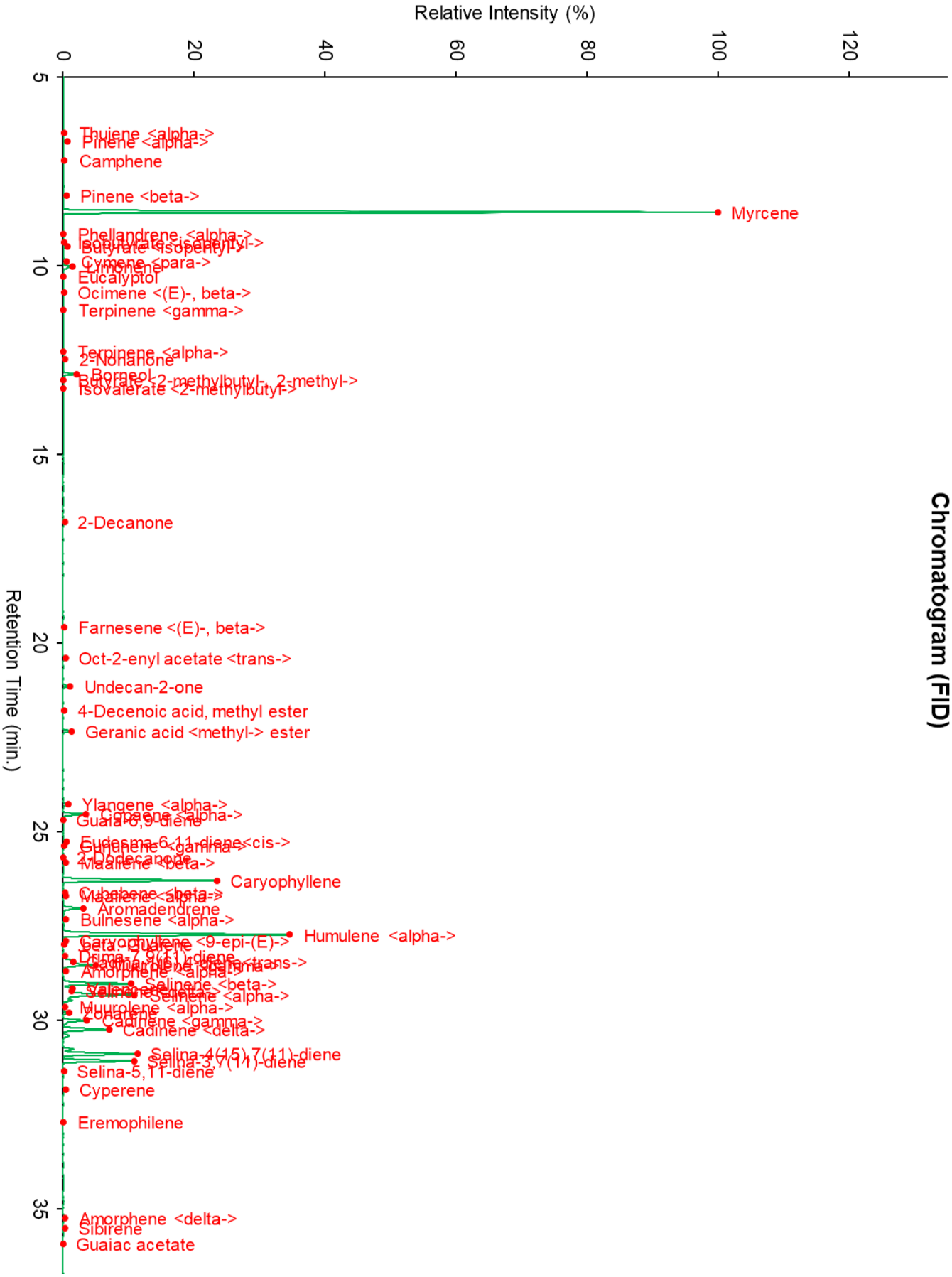
General Information – Example chromatogram of isomerized alpha-acids (HPLC-DAD)



General Information – Example chromatogram of xanthohumol (HPLC-DAD)



General Information – Example chromatogram of hops essential oil (GC-FID)



Extracts of hops *(Humulus lupulus)*, supercritical CO_2 (SFE) and subcritical water (SWE)
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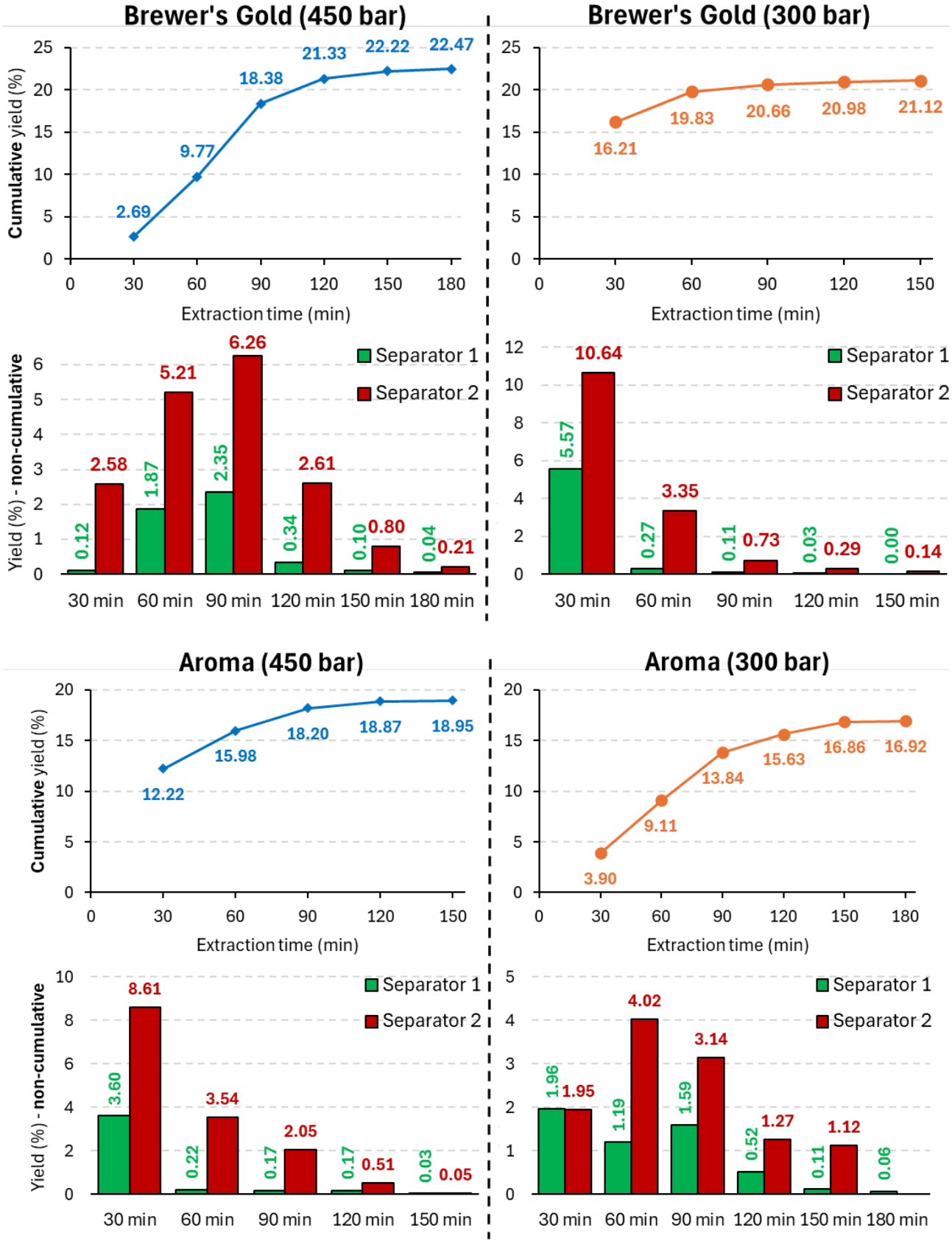
Results – Progression of bitter acid extraction (initial trial)

Initial trials at 300 and 450 bar with fractionation at 150 bar were carried out on two hop varieties, including **BG** and **AR**, to monitor the progression of overall extraction yield.



Extracts of hops *(Humulus lupulus)*, supercritical CO_2 (SFE) and subcritical water (SWE)
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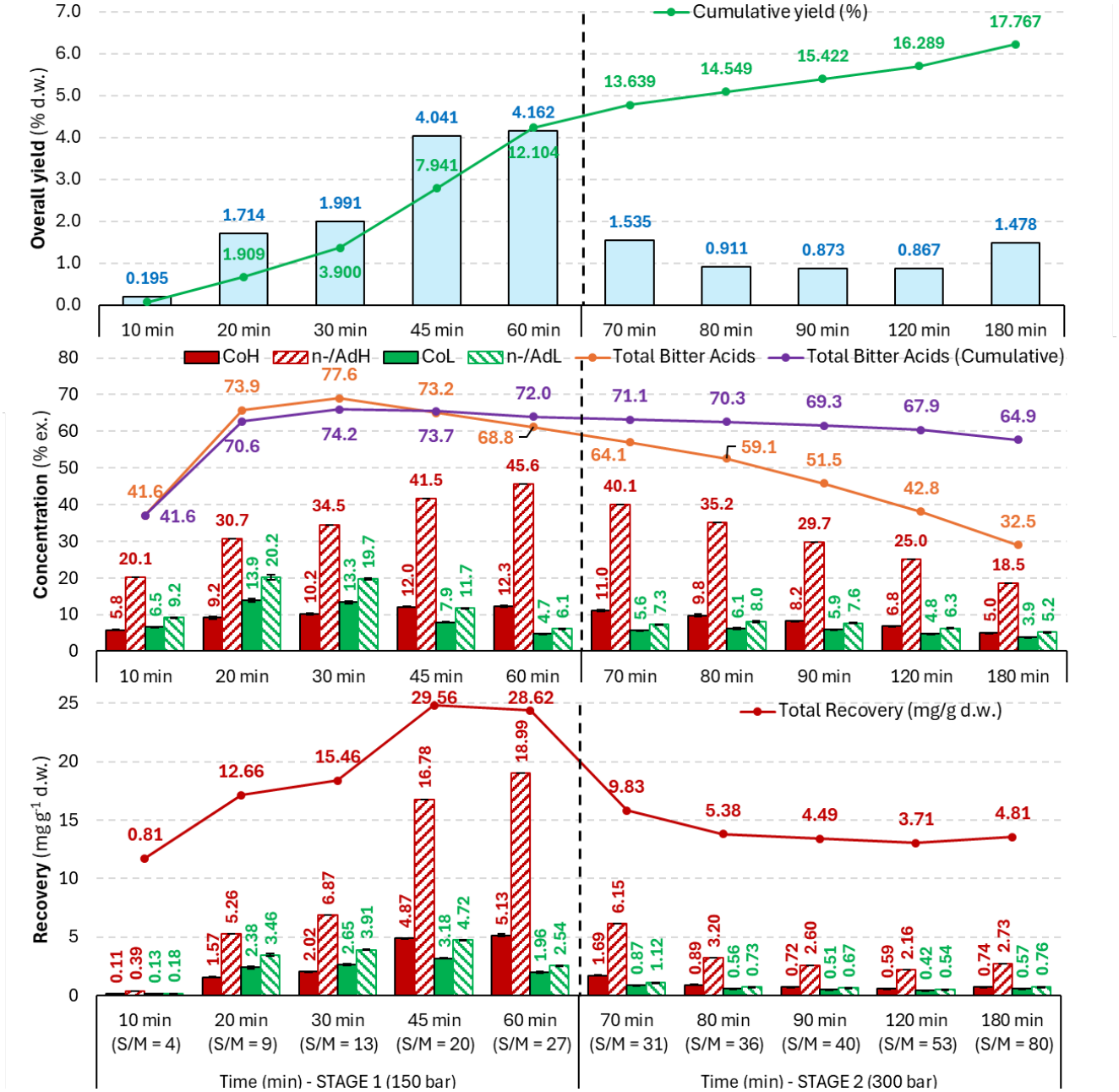
The results indicate that the majority of bitter acids are extracted in **120–150 min** at both 300 and 450 bar. Raising the pressure from 300 to 450 bar slightly increases the overall extraction yield (ca. **1.3%** and **2.0%** higher for **BG** and **AR**, respectively). Additionally, rapid extraction rate increase was observed at 450 bar for **AR** hops, such that **>65%** of extractable bitter acids obtained in the first **30 minutes**. However, this was not observed for **BG** hops, where **>93%** extraction was achieved in **60 minutes** at 300 bar instead. Therefore, the higher pressure was unlikely to be the main factor contributing to a quicker extraction – instead, both **AR** hops at 450 bar and **BG** hops at 300 bar were subjected to a **15-minute period of static extraction** at the start of the process, which appeared to have aided the dissolution of bitter acids prior to dynamic circulation of CO_2.

Results – Progression of bitter acid extraction at 150 bar (in-depth trial)

A second extraction trial was carried out on the **MG** bitter hop variety, which was extracted in **two stages** at **150 bar (60 min)** and **300 bar (120 min)** with regular extract collection every 10–60 minutes to determine whether extraction at 150 bar yields the complete bitter acid profile of the plant and whether raising the pressure to 300 bar results in a significantly different extract composition.



Extracts of hops *(Humulus lupulus)*, supercritical CO_2 (SFE) and subcritical water (SWE)
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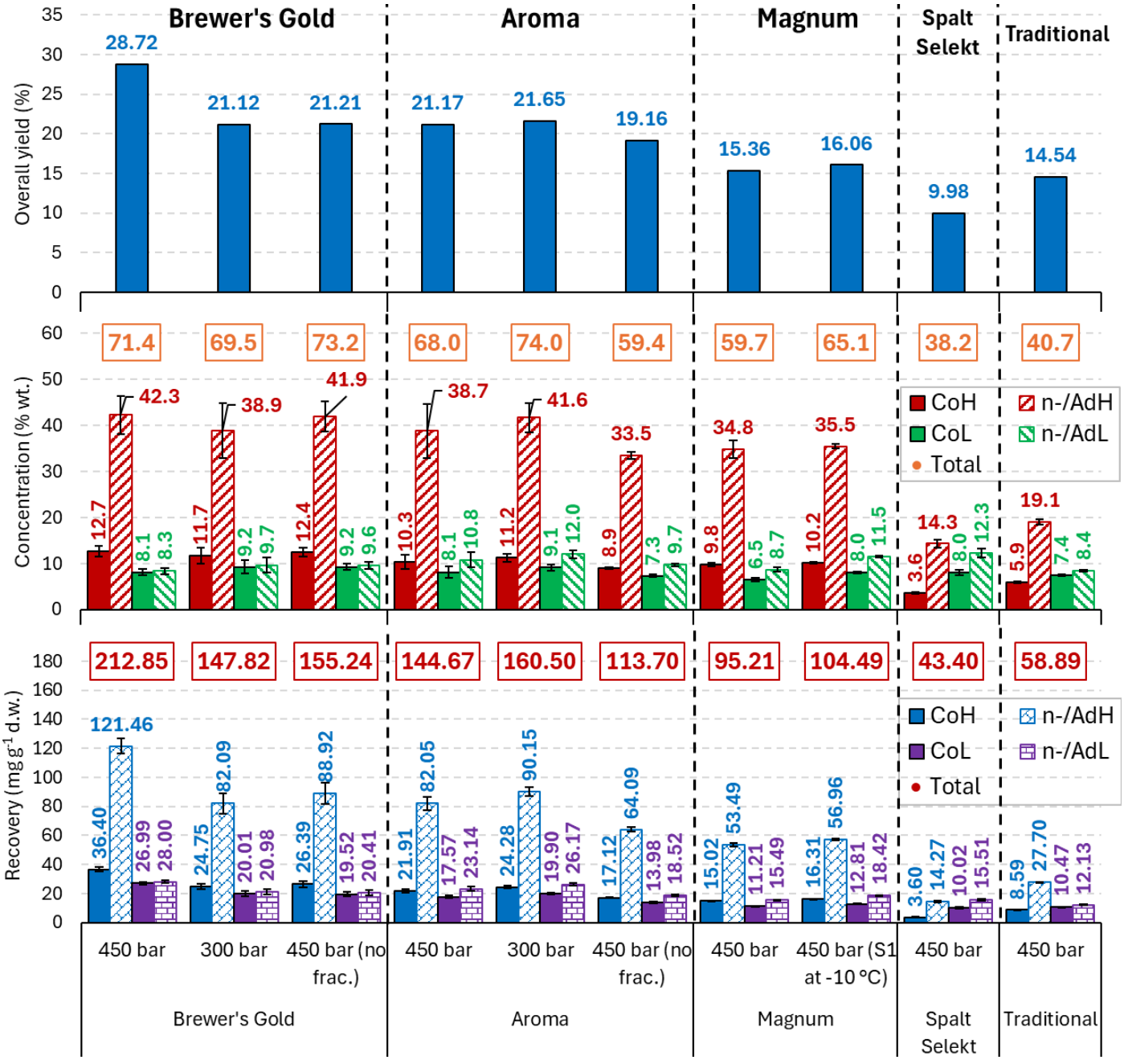
It is apparent that the extraction of both α- and β-acids is possible at 150 bar as well as 300 bar and that the relative composition of the acids does not change significantly between the two pressures, wich humulones always constituting the majority of the assemblage. Similarly to previous results, **>68%** of bitter acids are extracted in **60 minutes** at 150 bar, and **>91%** in a **further 60 minutes** at 300 bar. Thus, a total extraction time of 120 minutes is sufficient for practically complete recovery. The highest concentration and recovery occurs at 30 – 60 minutes of extraction, with bitter acids reaching >77% in the extract. Although the **concentration of bitter acids progressively decreases** in extract fractions collected after 30 minutes, the **cumulative (yield-corrected) concentration only decreases below 70% after 80–90 minutes, and is 68% after 120 minutes**. Overall, an hour of extraction at each pressure (150 bar and 300 bar) is sufficient for satisfactory extraction (total **S/M > 50**).

Results – The effect of extraction parameters on bitter acid profiles obtained from different hops

The effect of different extraction parameters (summarized in the table above) on bitter acid concentration and recovery from all **5 tested hop varieties** are summarized in the graph below.



Extracts of hops *(Humulus lupulus)*, supercritical CO_2 (SFE) and subcritical water (SWE)
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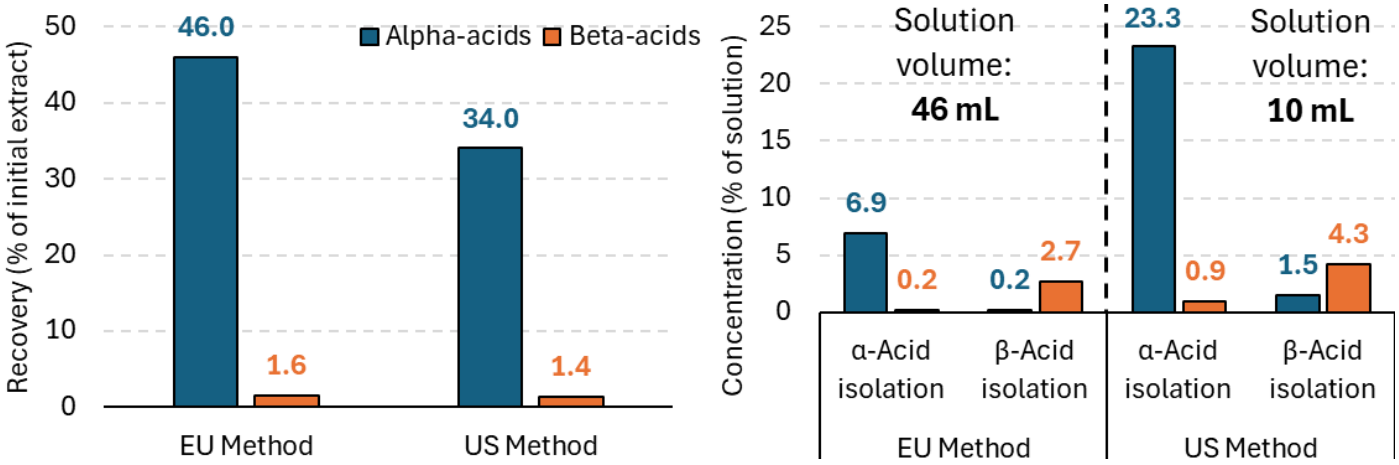
As expected, overall extraction yields and concentrations varied significantly among different hop varieties. The highest concentrations **>70%** were observed in **BG** and **AR** bitter hops, while **TD** and **SP** aromatic hops exhibited concentrations of ca. **≤40%** or less. The latter two were also characterized by the highest relative content of β-acids, which comprised **53%** and **39%** of total bitter acids for **SP** and **TD**, respectively. Comparatively low relative contents of β-acids was observed for **MG** (**33%**), **AR** (**29%**), and **BG** (**26%**) hops.

More dramatic differences were observed in total recoveries of bitter acids, with **>200 mg g^{-1}** recoverable from **BG** hops, while aromatic varieties yielded ca. **40–60 mg g^{-1}**. For **BG** hops a significant increase in overall yield and recovery from ca. **150 mg g^{-1}** to **>200 mg g^{-1}** was observed at 450 bar relative to 300 bar, while the same effect was not observed for **AR** hops, possibly due to matrix-specific effects. **Excluding fractionation at 150 bar** in separator 1 apparently **decreased bitter acid recovery**. For **MG** hops, cooling the first separator to -10 °C resulted in a significantly more free-flowing extract without a loss in overall yield and a >10% improvement in both bitter acid concentration and recovery.

*Results – Separation of α- and β-acids by refluxing with aqueous alkali (**TRIAL 1**)*

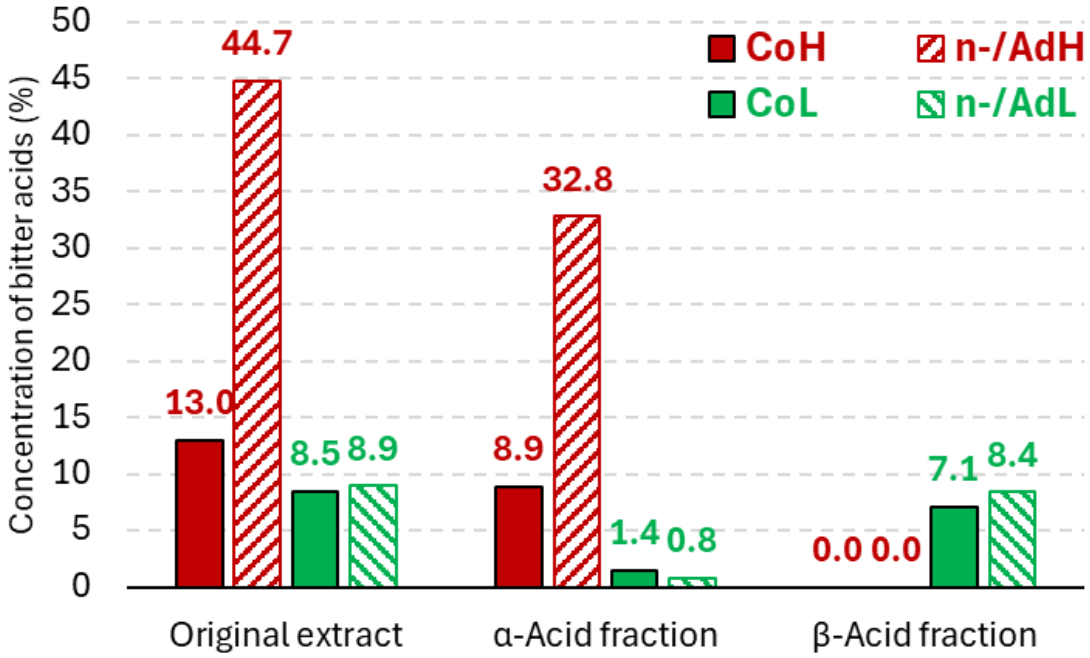
The crude extract of **MG** hops was subjected to two different methods of treatment with aqueous potassium hydroxide (KOH) in order to recover separate aqueous fractions of humulones and lupulones. In the first method (hereafter the **EU method**), a 15% concentration of α-acids (15 g of crude extract in 46 mL) was used with a 1.2 mol equivalent of KOH (thus requiring a 0.50 M solution). The solution was refluxed for **40 minutes at ca. 100 °C**. The second method (hereafter the **US method**), the solution was **agitated at 60 °C for 30 minutes** with 10 mL of a **1.78 M KOH solution** (a **1.92 mol equivalent**). The results are summarized below.



The results highlight the selectivity of both methods for recovering α-acids, with β-acids comprising **<4%** of total bitter acids in both aqueous extracts. However, only **46%** and **34%** of α-acids were recovered from the initial extract by both methods. Further trials are necessary to optimize the process. In case of the EU method, the relatively high amount of alpha acids left in the initial extract also caused their significant co-extraction into the subsequent β-acid fraction.

*Results – Separation of α- and β-acids by refluxing with aqueous alkali (**TRIAL 2**)*

During the second trial, the mol equivalent of KOH to alpha acids was more strictly controlled, setting the alpha acids concentration to **15%** *w/w* at a mol equivalent of **1.2** (using **385 mL** of a **0.5 M** aqueous KOH for ca. **100 g** of extract). The aqueous layer was concentrated, and the residue treated with an excess of alkali and also subsequently concentrated. The results are shown below.



It is clear that both alpha- and beta-acids were successfully concentrated in their respective fractions. While a small amount of beta-acids was retained in the alpha-acid concentrate, this could be further reduced with process optimization and/or a more extensive cleanup. The total recoveries (% of acids present in the original extract) of alpha- and beta-acids were **72%** and **49%**, respectively.

Results – Composition of essential oils recovered from whole extracts

Essential oils were recovered by distillation simultaneously with conversion of alpha-acids into their potassium salts. The composition of essential oil was determined by GC-MS and GC-FID.

GC-MS results

Analyte name	CAS	Chemical Class	RT* (min)	Content (%)
Thujene <alpha->	2867-05-2	Monoterpene	4.796	0.06
Pinene <alpha->	80-56-8	Monoterpene	4.962	0.22
Camphene	79-92-5	Monoterpene	5.342	0.06
Pinene <beta->	127-91-3	Monoterpene	6.131	0.18
Myrcene	123-35-3	Monoterpene	6.629	31.39
Phellandrene <alpha->	99-83-2	Monoterpene	6.993	0.04
Isobutyrate <isopentyl->	2050-01-3	Non-terpenoid Ester	7.334	0.04
Butyrate <isopentyl->	106-27-4	Non-terpenoid Ester	7.449	0.17
Cymene <para->	99-87-6	Monoterpenoid Alkene	7.663	0.11
Limonene	138-86-3	Monoterpene	7.81	0.8
Eucalyptol	470-82-6	Monoterpenoid Oxide	7.89	0.03
Ocimene <(E)-, beta->	3779-61-1	Monoterpene	8.562	0.03
Terpinene <gamma->	99-85-4	Monoterpene	8.903	0.03
Terpinene <alpha->	99-86-5	Monoterpene	10.005	0.03
2-Nonanone	821-55-6	Non-terpenoid Ketone	10.214	0.06
Borneol	51117-20-5	Monoterpenoid Ester	10.495	0.53
Butyrate <2-mbutyl-, 2-methyl->	2445-78-5	Ester	10.7	0.03

Analyte name	CAS	Chemical Class	RT* (min)	Content (%)
Isovalerate <2-methylbutyl->	2445-77-4	Ester	10.882	0.02
2-Decanone	693-54-9	Ketone	14.34	0.06
Farnesene <(E)-, beta->	18794-84-8	Sesquiterpene	16.939	0.03
Oct-2-enyl acetate <trans->	3913-80-2	Ester	17.872	0.06
Undecan-2-one	112-12-9	Non-terpenoid Ketone	18.603	0.22
4-Decenoic acid, methyl ester	1191-02-2	FAME	19.277	0.03
Geranic acid <methyl-> ester	1189-09-9	Monoterpenoid Ester	19.858	0.23
Ylangene <alpha->	14912-44-8	Sesquiterpene	21.659	0.4
Copaene <alpha->	138874-68-7	Sesquiterpene	21.845	1.57
Guaia-6,9-diene	37839-64-8	Sesquiterpene	21.978	0.04
Eudesma-6,11-diene<cis->	194607-93-7	Sesquiterpene	22.484	0.22
Gurjunene <gamma->	22567-17-5	Sesquiterpene	22.65	0.07
2-Dodecanone	6175-49-1	Ketone	22.81	0.02
Maaliene <beta->	97408-24-7	Sesquiterpene	23.206	0.2
Caryophyllene	87-44-5	Sesquiterpene	23.57	9.44
Cubebene <beta->	13744-15-5	Sesquiterpene	23.979	0.21
Maaliene <alpha->	489-28-1	Sesquiterpene	24.15	0.09
Aromadendrene	109119-91-7	Sesquiterpene	24.343	1.43
Bulnesene <alpha->	489-81-6	Sesquiterpene	24.536	0.16
Humulene <alpha->	6753-98-6	Sesquiterpene	24.941	15.36
Caryophyllene <9-epi-(E)->	68832-35-9	Sesquiterpene	25.207	0.17
.beta.-Guaiene	88-84-6	Sesquiterpene	25.303	0.35
Drima-7,9(11)-diene	872795-35-2	Sesquiterpene	25.581	0.13
Cadina-1(6),4-diene<trans->	20085-11-4	Sesquiterpene	25.8	0.44
Muurolene <gamma->	30021-74-0	Sesquiterpene	25.932	3.2
Amorphene <alpha->	483-75-0	Sesquiterpene	26.067	0.23
Selinene <beta->	17066-67-0	Sesquiterpene	26.226	4.75
Valencene	4630-07-3	Sesquiterpene	26.315	0.06
Selinene <delta->	473-14-3	Sesquiterpene	26.505	0.05
Selinene <alpha->	473-13-2	Sesquiterpene	26.599	6.43
Muurolene <alpha->	10208-80-7	Sesquiterpene	26.883	1.39
Zonarene	41929-05-9	Sesquiterpene	27.144	0.17
Cadinene <gamma->	38357-83-4	Sesquiterpene	27.373	2
Hinesol acetate	88494-77-3	Sesquiterpenoid Ester	27.562	1.04
Cadinene <delta->	16729-01-4	Sesquiterpene	27.794	3.78
Selina-4(15),7(11)-diene	58893-88-2	Sesquiterpene	28.137	5.61
Selina-3,7(11)-diene	222187-60-2	Sesquiterpene	28.383	6.12
Selina-5,11-diene	52026-55-8	Sesquiterpene	28.54	0.09
Cyperene	2387-78-2	Sesquiterpene	29.236	0.19
Eremophilene	72747-25-2	Sesquiterpene	29.957	0.02
Amorphene <delta->	317819-82-2	Sesquiterpene	31.665	0.03
Sibirene	14029-18-6	Sesquiterpene	31.774	0.07
Guaiac acetate	134-28-1	Sesquiterpenoid Ester	32.137	0.04
		TOTAL IDENTIFIED (%):		**100**

Extracts of hops *(Humulus lupulus)*, supercritical CO_2 (SFE) and subcritical water (SWE)
This report was prepared for -.

GC-FID results

Analyte name	CAS	Chemical Class	RT* (min)	Content (%)
Thujene <alpha->	2867-05-2	Monoterpene	6.474	0.05
Pinene <alpha->	80-56-8	Monoterpene	6.704	0.20
Camphene	79-92-5	Monoterpene	7.219	0.07
Pinene <beta->	127-91-3	Monoterpene	8.138	0.18
Myrcene	123-35-3	Monoterpene	8.583	33.14
Phellandrene <alpha->	99-83-2	Monoterpene	9.153	0.03
Isobutyrate <isopentyl->	2050-01-3	Non-terpenoid Ester	9.377	0.06
Butyrate <isopentyl->	106-27-4	Non-terpenoid Ester	9.499	0.23
Cymene <para->	99-87-6	Monoterpenoid Alkene	9.882	0.27
Limonene	138-86-3	Monoterpene	10.022	0.65
Eucalyptol	470-82-6	Monoterpenoid Oxide	10.296	0.02
Ocimene <(E)-, beta->	3779-61-1	Monoterpene	10.71	0.06
Terpinene <gamma->	99-85-4	Monoterpene	11.167	0.03
Terpinene <alpha->	99-86-5	Monoterpene	12.274	0.05
2-Nonanone	821-55-6	Non-terpenoid Ketone	12.487	0.10
Borneol	51117-20-5	Monoterpenoid Ester	12.871	0.77
Butyrate <2-mbutyl-, 2-methyl->	2445-78-5	Ester	13.031	0.06
Isovalerate <2-methylbutyl->	2445-77-4	Ester	13.253	0.04
2-Decanone	693-54-9	Ketone	16.799	0.14
Farnesene <(E)-, beta->	18794-84-8	Sesquiterpene	19.587	0.09
Oct-2-enyl acetate <trans->	3913-80-2	Ester	20.395	0.16
Undecan-2-one	112-12-9	Non-terpenoid Ketone	21.145	0.42
4-Decenoic acid, methyl ester	1191-02-2	FAME	21.796	0.08
Geranic acid <methyl-> ester	1189-09-9	Monoterpenoid Ester	22.336	0.52
Ylangene <alpha->	14912-44-8	Sesquiterpene	24.274	0.38
Copaene <alpha->	138874-68-7	Sesquiterpene	24.538	1.41
Guaia-6,9-diene	37839-64-8	Sesquiterpene	24.697	0.03
Eudesma-6,11-diene<cis->	194607-93-7	Sesquiterpene	25.267	0.25
Gurjunene <gamma->	22567-17-5	Sesquiterpene	25.384	0.13
2-Dodecanone	6175-49-1	Ketone	25.685	0.04
Maaliene <beta->	97408-24-7	Sesquiterpene	25.813	0.20
Caryophyllene	87-44-5	Sesquiterpene	26.296	9.45
Cubebene <beta->	13744-15-5	Sesquiterpene	26.61	0.14
Maaliene <alpha->	489-28-1	Sesquiterpene	26.705	0.21
Aromadendrene	109119-91-7	Sesquiterpene	27.033	1.37
Bulnesene <alpha->	489-81-6	Sesquiterpene	27.33	0.19
Humulene <alpha->	6753-98-6	Sesquiterpene	27.733	14.81
Caryophyllene <9-epi-(E)->	68832-35-9	Sesquiterpene	27.891	0.23
.beta.-Guaiene	88-84-6	Sesquiterpene	27.98	0.07
Drima-7,9(11)-diene	872795-35-2	Sesquiterpene	28.296	0.15
Cadina-1(6),4-diene<trans->	20085-11-4	Sesquiterpene	28.454	1.01
Muurolene <gamma->	30021-74-0	Sesquiterpene	28.539	2.33
Amorphene <alpha->	483-75-0	Sesquiterpene	28.701	0.22
Selinene <beta->	17066-67-0	Sesquiterpene	29.033	4.50

Extracts of hops *(Humulus lupulus)*, supercritical CO_2 (SFE) and subcritical water (SWE)

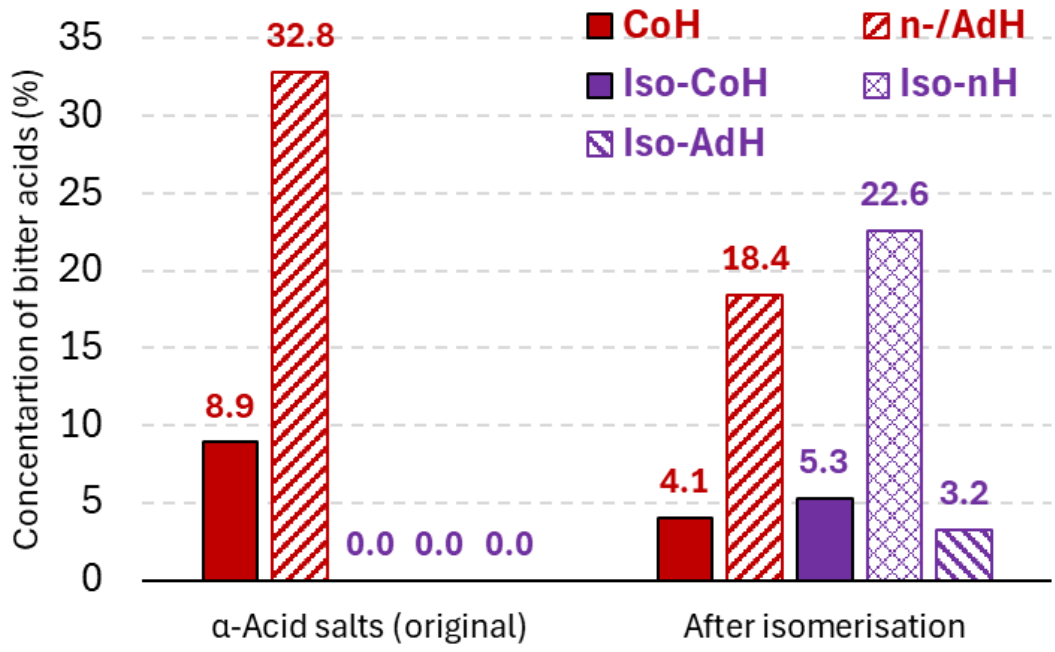
This report was prepared for -.

Analyte name	CAS	Chemical Class	RT* (min)	Content (%)
Valencene	4630-07-3	Sesquiterpene	29.153	0.64
Selinene <delta->	473-14-3	Sesquiterpene	29.222	0.48
Selinene <alpha->	473-13-2	Sesquiterpene	29.331	5.31
Muurolene <alpha->	10208-80-7	Sesquiterpene	29.643	0.19
Zonarene	41929-05-9	Sesquiterpene	29.806	0.38
Cadinene <gamma->	38357-83-4	Sesquiterpene	30.01	1.75
Cadinene <delta->	16729-01-4	Sesquiterpene	30.246	3.27
Selina-4(15),7(11)-diene	58893-88-2	Sesquiterpene	30.894	4.89
Selina-3,7(11)-diene	222187-60-2	Sesquiterpene	31.079	4.31
Selina-5,11-diene	52026-55-8	Sesquiterpene	31.351	0.08
Cyperene	2387-78-2	Sesquiterpene	31.839	0.24
Eremophilene	72747-25-2	Sesquiterpene	32.694	0.04
Amorphene <delta->	317819-82-2	Sesquiterpene	35.249	0.32
Sibirene	14029-18-6	Sesquiterpene	35.512	0.15
Guaiac acetate	134-28-1	Sesquiterpenoid Ester	35.925	0.05
			TOTAL QUANTIFIED (%):	**96.63**

The ratio of myrcene to humulene in these oils is a **desirable 2:1**, while the yield is a typical **1.7–2.2 %**, showing that oil recovery by simple distillation following extraction with supercritical CO_2 is a viable option for its separation.

Results – Isomerization of α-acids by heating

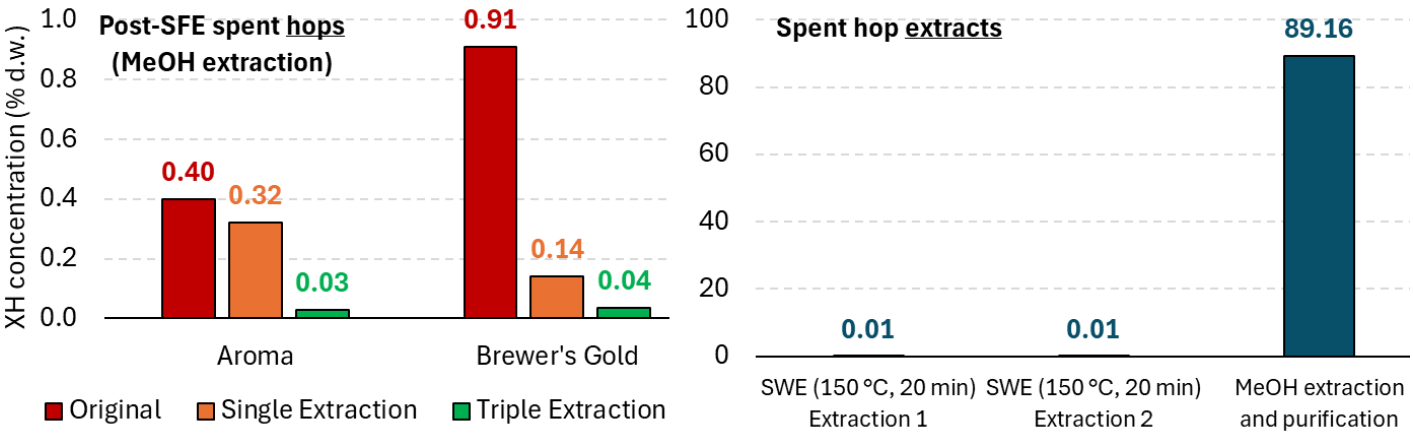
The concentrated alpha-acid fraction from the previous trial was isomerized by boiling the aqueous solution for 6 hours. The results are reported below.



Alpha acids were successfully isomerized, but the process was incomplete at only a **48% conversion rate**. The process could be easily improved and the rate of isomerization increased by employing temperatures **>100 °C** (e.g. 120–140 °C) under pressure, but this was not attempted in this study.

Results – Xanthohumol extraction and purification from spent hops

The extraction of xanthohumol was attempted using subcritical water (SWE) at 150 °C and a more traditional methanol extraction followed by purification of diatomaceous earth. The methanol extraction was carried out either once or repeated three times, and the xanthohumol content in the residues re-measured to determine the thoroughness of xanthohumol recovery. Similarly, the concentration of xanthohumol in SWE and methanol extracts was measured. The results are summarized below.

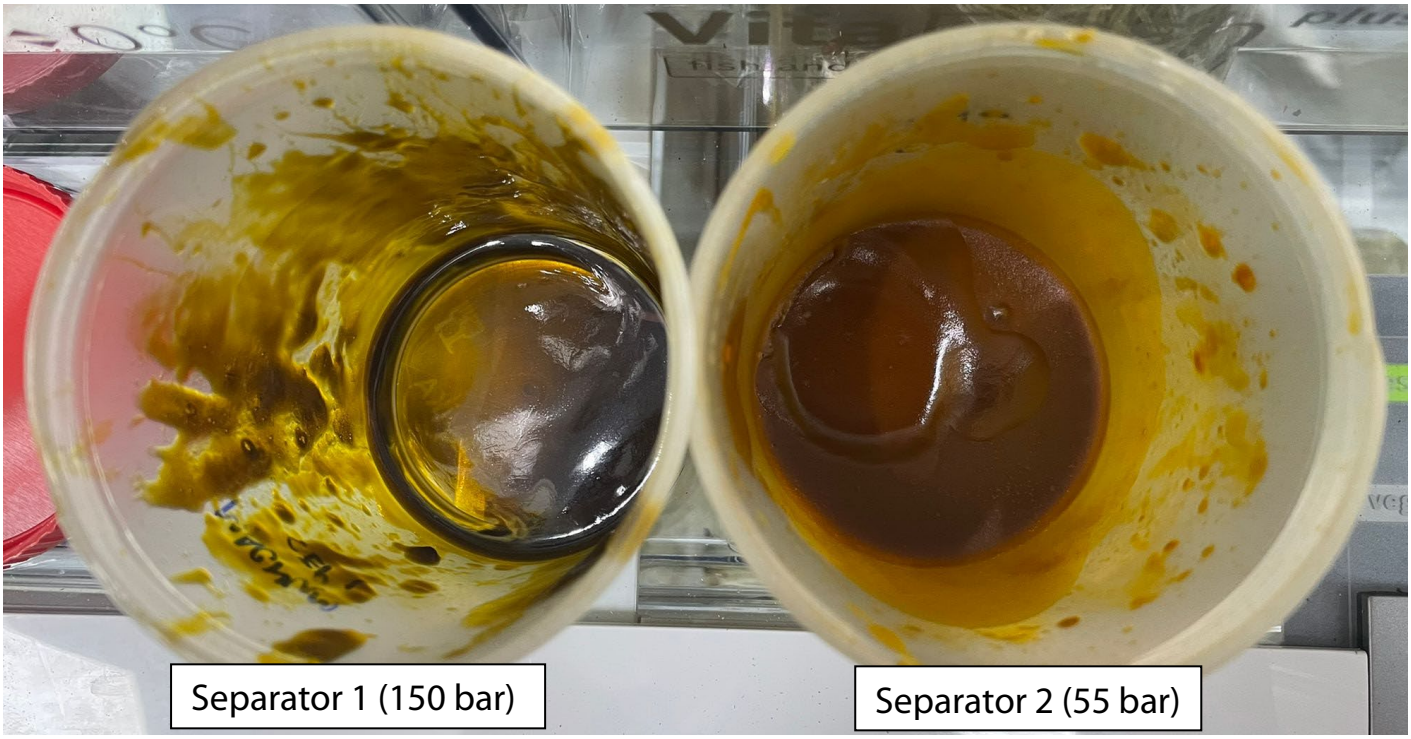


Triple extraction dramatically improves recovery of xanthohumol, while the resulting xanthohumol concentration after purification is **>89%**, and is likely to improve with recrystallization (not attempted here but well documented in the literature). In contrast, SWE yields near-zero xanthohumol, either due to reduced selectivity of extraction or (more likely) thermal degradation or isomerization.

Main outcomes

- Extraction at **300–450 bar** with **fractionation at 150 bar** in separator 1 extracts all bitter acids at **S/M > 50**.
- Alternatively, separator 1 may be cooled to **-10 °C** to precipitate cuticular waxes without loss of bitter acids.
- Raising pressure from **300 to 450 bar** may facilitate a quicker and more complete extraction in **some** hops.
- Treatment of the crude SFE extract with aqueous alkali successfully separates α- and β-acids, but strict control of process conditions is required. It is possible to achieve an **alpha-acid content comprising >95% of total bitter acids**.
- Essential oils can be completely recovered during the conversion of alpha-acids to their potassium salts. The ratio of myrcene to humulene in the oil is characteristic at **2:1–3:1**.
- Isomerization of isolated alpha-acids is possible through simple application of heat. Application of pressure and temperature in excess of 100 °C may be useful to accelerate the process and improve the conversion rate.
- Extraction of xanthohumol from spent hops is most successful with methanol with simple purification via diatomaceous earth. Purities of **ca. 90%** are achievable even before recrystallisation. SWE extraction likely results in thermal degradation.

Extract photographs



Extracts of hops *(Humulus lupulus)*, supercritical CO_2 (SFE) and subcritical water (SWE)
This report was prepared for -.

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