

Supercritical fluid extraction of hops

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Abstract: Five cultivars of hop were extracted by the method of supercritical fluid extraction using carbon dioxide (SFE–CO₂) as extractant. The extraction (50 g of hop sample using a CO₂ flow rate of 97.725 L/h) was done in the two steps: 1. extraction at 150 bar and 40°C for 2.5 h (sample of series A was obtained) and, after that, the same sample of hop was extracted in the second step: 2. extraction at 300 bar and 40 °C for 2.5 h (sample of series B was obtained). The Magnum cultivar was chosen for the investigation of the extraction kinetics. For the qualitative and quantitative analysis of the obtained hop extracts, the GC-MS method was used. Two of the most common compounds of hop aroma (α -humulene and β -caryophyllene) were detected in samples of series A. In addition, isomerized α -acids and a high content of β -acids were detected. The α -acids content in the samples of series B was the highest in the extract of the Magnum cultivar (it is a bitter variety of hop). The low contents of α -acids in all the other hop samples resulted in extracts with low α -acids content, *i.e.*, that contents were under the prescribed α -acids content.

Keywords: *Humulus lupulus*, Cannabinaceae, hop extraction, supercritical carbon dioxide.

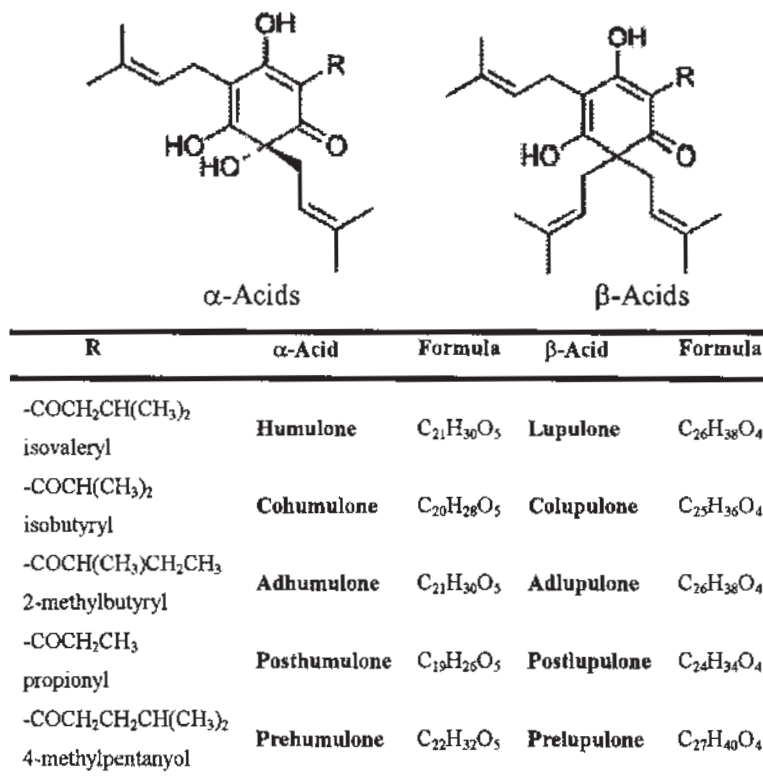
INTRODUCTION

In the production of beer, hop is an essential component. The aroma and bitterness compounds of hop are the most important for brewers. Over 300 compounds of hop essential oil (aroma components) have been detected to date. These compounds are mainly hydrocarbons, ketones, aldehydes, esters, carboxylic acids, alcohols, oxygen heterocyclic and sulfur compounds. The hydrocarbons, as terpenes (usually content of 40-80% in hop essential oil), are the most important.¹ The contents of myrcene, α -humulene, β -caryophyllene and β -farnesene, as well as their ratio, are distinctive for a certain variety.² The bitter compounds (α - and β -acids) are shown in Fig. 1.

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Fig. 1. α -Acids and β -acids of hop.

The different contents of bitter compounds is characteristic for different hop varieties. Depending on the α -acids content, the two categories are aromatic (3.8 – 5.1 %) and bitter hop (8.6 – 12.9 % of α -acids).¹

These compounds may degrade or evaporate during storage, especially if the storage conditions are unsuitable. By using hop extracts obtained by SFE-CO₂ instead raw hop, many industrial problems could be resolved (such are energy saving, no cooling, no storage problems, extract is stable at room temperature, *etc.*).³

SFE Extraction of hops, as well as decaffeination of coffee and nicotine extraction from tobacco, was one of the first scientific interests of applying this modern method of extraction on the industry scale.⁴⁻⁷

For the separation of the aromatic and bitter fraction of hop, supercritical fluid extraction using carbon dioxide (SFE-CO₂) in two extraction steps could be used. The extract containing mainly essential oil compounds could be obtained at a lower solubility power of CO₂ (such as a pressure of 150 bar and temperature of 40 °C, *i.e.*, a solvent density of 0.790 g/cm³), and subsequently increasing the solubility power (300 bar, 40 °C, solvent density of 0.915 g/cm³), for the second extract, *i.e.*, the extract which contains the bitter hop compounds.

EXPERIMENTAL

Plant material. All hop cultivars were produced by the Institute of Hop, Broomcorn and Medical plants, Bački Petrovac, Serbia (year 1999). The samples were dried cones of hop. Before extraction, all the hop samples were milled to a mean particle radius of 0.448×10^{-3} m.

Chemicals. Commercial carbon dioxide (Tehno-gas, Novi Sad, Serbia) was used as the extracting agent for supercritical fluid extraction (SFE). All other chemicals were of analytical reagent grade.

Sample preparation. The procedure of obtained samples was:

1. The essential oil content was determined using the Ph. Jug. IV procedure.⁸
2. The yield of the total extract (TE) was obtained by Soxhlet extraction using *n*-hexane.
3. Extracts were obtained by SFE-CO₂. The investigated hop sample (50.0 g) was extracted by carbon dioxide at a temperature of 40 °C and a CO₂ flow rate of 97.725 L/h, in two steps, at 150 bar and 300 bar, respectively. The extraction time for each step was 2.5 h. The separation conditions were $p = 15 \pm 1$ bar and $T = 20 \pm 1$ °C.

The SFE-CO₂ was carried out using a laboratory-scale, high pressure extraction plant (NOVA-Swiss, Effretikon, Switzerland) described previously.⁹ The main parts and characteristics (manufacturer specification) of the plant are as follows: a diaphragm-type compressor (up to 1000 bar) extractor with an internal volume of 200 mL ($p_{\max} = 700$ bar), a separator with an internal volume of 200 mL ($p_{\max} = 250$ bar) and a maximum CO₂ mass flow rate of approximately 5.7 kg/h.

GC-MS. A GCD HP G 1800 A (Hewlett-Packard, Palo Alto, Calif, USA) instrument with a HP-5 MS column (30.0 m x 0.25 mm; film thickness 0.25 µm) was used. The helium flow rate was 0.8 mL/min. The injector temperature was 250 °C; the detector was set at 280 °C, it was initially set at 50 °C and then increased linearly at 20 °C per min to 130 °C held for 1 min and then increased at 9 °C per min until the final temperature of 280 °C (8.33 min) was attained. The total analysis time was 30 min. The injected volume of sample solution in dichloromethane – diethylether mixture in a ratio 7 : 3 (10 mg/mL) was 5 µL (splitless injection). The mass spectrum was obtained using the SCAN-technique at the interval of 45–425 a.m.u. The compounds were identified using the Wiley database.

Conductometric titration. Analysis of extracts obtained at 300 bar was done by the conductivity titration method modified by Wöllmer.¹⁰

RESULTS AND DISCUSSION

The content of hop essential oil was determined by an official procedure.⁸ The total extract (TE) yield was determined by Soxhlet extraction with *n*-hexane. In order to prevent the thermal decomposition of hop compounds, the temperature of 40 °C was selected for the SFE-CO₂. Two steps were used for the SFE-CO₂ to obtain extracts containing aroma and bitterness hop compounds: SFE-CO₂ extraction of hop at 150 bar, *i.e.*, a solvent density of 0.790 g/cm³, for 2.5 h (Samples of series A were obtained) and, subsequently, the same sample of hop was extracted at 300 bar,

TABLE I. Results of hop extraction

Cultivar	Magnum	Hallertau Tradition	Spalt Selekt	Aroma	K-62
Essential oil content/%; ml/100 g	1.35	0.25	0.58	0.25	0.55
TE yield/%; g/100 g	34.7	16.5	16.9	11.2	17.2
CO ₂ -extract yield at 150 bar/%; g/100 g*	13.35	6.18	9.09	7.04	6.54
CO ₂ -extract yield at 300 bar/%; g/100 g**	7.54	2.46	2.31	3.59	2.90

*Samples of series A; **Samples of series B

i.e., a solvent density of 0.915 g/cm^3 , for 2.5 h (Samples of series B were obtained). All these results are shown in Table I.

The essential oil content was the highest in the Magnum cultivar (1.35 %). This content could vary from 0.5 to 2.5 %.¹¹ Samples of Hallertay Tradition and Aroma showed a low content of essential oil. The TE yield of Magnum (34.7 %) was much higher than that of all the other investigated hop cultivars, *i.e.*, the TE yield was twice lower for the samples than for Magnum. This conclusion was the same for SFE-CO₂ of hop. The CO₂-extract yield obtained at 150 bar, as well as at 300 bar, was much higher for Magnum (13.35 % and 7.54 %, respectively) than those for all the other hop samples (from 6.18 % to 9.09 % and from 2.31 % to 3.59 %, respectively).

TABLE II. SFE-CO₂ extraction kinetics of hop Magnum

Extraction time/min	Ratio $m_{\text{CO}_2}/m_{\text{hop}}$ g/g	Yield at 150 bar %; g/100 g	Yield at 300 bar %; g/100 g
15	0.967	1.71	1.68
30	1.935	4.38	3.38
45	2.901	6.63	4.22
60	3.868	8.16	5.13
90	5.803	10.47	6.19
120	7.736	11.99	6.86
150	9.671	13.35	7.54

(Samples of series A) (Samples of series B)

On the basis of the obtained results the Magnum cultivar was selected for investigation of the extraction kinetics of hop by SFE-CO₂ (Table II and Fig. 3).

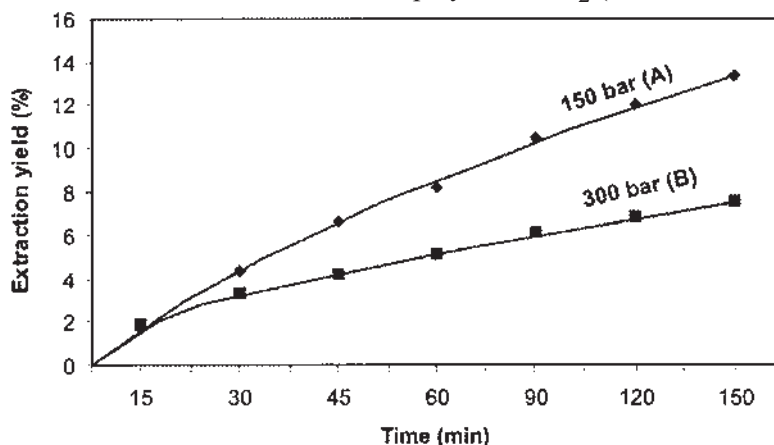


Fig. 2. Extraction kinetics of Magnum by SFE-CO₂.

The content of α -acids in the extracts by SFE-CO₂ (sample of series B), as well as in the investigated native hops, was determined using the conductometric titration method (Table III).

TABLE III. α -Acid content in native hope and extract obtained by SFE-CO₂ (Samples of series B)

Hop cultivar	α -Acids content/%	
	Native hop	Extract of series B
Magnum	14.7	41.0
Hallertau Tradition	4.0	16.9
Spalt Selekt	4.7	9.8
Aroma	6.3	11.6
K-62	3.3	9.9

The native sample of Magnum cultivar (bitter variety of hop) had highest content of α -acids. After separation of the aroma fraction of hop using SFE-CO₂ at extraction conditions of 150 bar, 40 °C and 2.5 h, the samples were extracted at a higher solubility power of CO₂ (300 bar and same temperature of 40 °C). In this way, after 2.5 h of extraction, hop extracts, *i.e.*, samples of series B, were obtained. These samples shows, of course, a higher content of α -acids than the native hop samples and, again, the highest α -acis content in CO₂-extract were obtained for Magnum hop cultivar (41.0 %).

The main essential oil compounds of Magnum hop (essential oil content of 1.35 %) were determined by GC-MS. The predominant compound of this essential oil was α -humulene (content in essential oil of 45.50 %). Myrcene (15.12 %), β -caryophyllene (12.54 %) and β -farnesene (1.42 %) were also detected and determined.

The GC-MS method was used for the qualitative and quantitative determination of compounds contained in the extracts obtained in the first step of SFE-CO₂ (150 bar, 40 °C and 2.5 h). The results of the content of compounds in these extracts (Samples of series A) of the investigated hop types are shown in Table IV. As an illustration, the GC-chromatogram of the Magnum extract is given in Fig. 3.

TABLE IV. GC-MS Results of CO₂ extraction of the investigated hop types (Samples of series A)

Retention time t_R /min	Compound	Compound percent area (%) in CO ₂ extract of following hop types				
		Magnum	Hallertau Tradition	Spalt Selekt	Aroma	K-62
8.88	β -Caryophyllene	2.21	tr.	0.16	0.09	0.10
9.33	α -Humulene	10.35	0.47	0.88	0.50	0.79
9.57	β -Kubebene	–	tr	0.37	0.06	–
10.07	Calarene	–	0.05	–	0.08	0.05
10.16	δ -Cadinene	–	0.14	–	0.13	0.15
18.83	Isohumulone	12.15	8.04	7.64	5.80	4.75
19.74	Colupulone	25.31	8.64	27.22	11.39	34.38
20.37	Adlupulone	6.93	4.30	8.36	8.54	8.13
20.45	Lupulone	36.37	10.84	29.67	4.50	8.64

$t_R < 0.05$ %

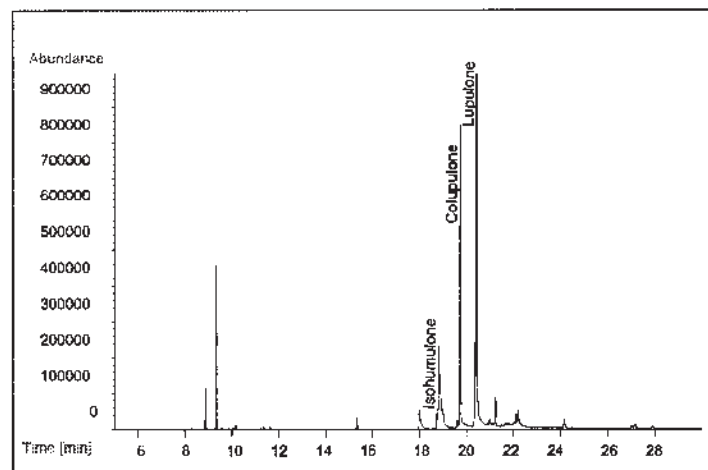


Fig. 3. GC-Chromatogram of Magnum extract obtained by SFE-CO₂ (Sample of series A).

The predominant compounds of the Magnum extract were lupulone (36.37 %), colupulone (25.31 %) and isohumulone (12.15 %). The content of the same compounds in Hallertau Tradition extract were much lower (10.84 %, 8.64 % and 8.04 %, respectively). The main compounds in the Spalt Selekt extract were lupulone (29.67 %), colupulone (27.22 %), adlupulone (8.36 %) and isohumulone (7.64 %). Colupulone (11.39 %) and adlupulone (8.54 %) have the highest content in the Aroma extract. The main compound of the K-62 extract was colupulone (34.38 %), while the contents of lupulone (8.64 %) and adlupulone (8.13 %) were lower. An α -humulene – β -caryophyllene ratio higher than 3 suggests an aromatic hop variety or a hop with a high-quality aroma. This ratio was higher than 3 for all the investigated CO₂-extracts (for Magnum, it was 4.7), but on the basis of the contents of the compounds, only hop of the Magnum cultivar could be classified as an aromatic hop variety, as well as bitter type of hop.

On the basis of these results, as well as of all results (Table IV), it could be concluded that the extracts of the investigated hop varieties are different in terms of their qualitative and quantitative composition. This fact is very important for selecting the best product (for many reasons, hop extracts obtained by SFE-CO₂) are suggested for the beer industry.

ИЗВОД

ЕКСТРАКЦИЈА ХМЕЉА СУПЕРКРИТИЧНИМ ФЛУИДОМ

ЗОРАН ЗЕКОВИЋ,* ИВАНА ПФАФ-ШОВЉАНСКИ, ОЛГИЦА ГРУЈИЋ

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Пет сорти хмеља је екстраховано применом методе суперкритичне флуид екстракције (SFE) и угљендиоксида (CO₂) као екстрагенса. Екстракција (50,0 g хмеља при

протоку CO₂ од 97.725 L/h) је вршена у два степена: 1. Екстракција на 150 бар и 40 °C у времену од 2,5 h (добијен је узорак серије А) и, након тога, исти узорак хмеља је екстрахован при следећим условима: 2. Екстракција на 300 бар и 40 °C у времену од 2,5 h (добијен је узорак серије В). Сорта хмеља Magnum је одабрана за испитивање кинетике екстракције. За квалитативну и квантитативну анализу добијених екстраката хмеља примењена је GC-MS метода. У узорцима серије А су детектоване две (α -хумулен и β -кариофилен) од четири уобичајене компоненте ароме хмеља. Осим тога, изомеризоване α -киселине и висок садржај β -киселина су детектовани у узорцима серије А. Садржај α -киселина у узорцима серије В је био највећи у екстракту сорте Magnum (припада горким сортама хмеља). Мали садржај α -киселина у свим осталим узорцима хмеља, односно у њиховим екстрактима, је испод вредности која је прописана за садржај α -киселина у хмељу.

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