

SUPERCRITICAL CARBON DIOXIDE HOP EXTRACTION

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The hop of Magnum cultivar was extracted using supercritical carbon dioxide (SFE-CO₂) as extractant. Extraction was carried out in the two steps: the first one being carried out at 150 bar and 40°C for 2.5 h (Extract A), and the second was the extraction of the same hop sample at 300 bar and 40°C for 2.5 h (Extract B). Extraction kinetics of the system hop-SFE-CO₂ was investigated. Two of four most common compounds of hop aroma (α -humulene and β -caryophyllene) were detected in Extract A. Isomerised α -acids and β -acids were detected too. α -Acid content in Extract B was high (that means it is a bitter variety of hop). Mathematical modeling using empirical model, characteristic time model and simple single sphere model has been performed on Magnum cultivar extraction experimental results. Characteristic time model equations, best fitted experimental results. Empirical model equation, fitted results well, while simple single sphere model equation poorly approximated the results.

KEY WORDS: Hop; supercritical extraction; mathematical modeling

INTRODUCTION

Hop is an essential component in beer production. Aroma and bitterness compounds are the most important for brewer. Over 300 compounds of hop essential oil (aroma components) has been detected till now. These compounds are namely hydrocarbons, ketones, aldehydes, esters, carboxylic acids, alcohols, oxygen heterocyclic, and sulfur compounds. The hydrocarbons, like terpenes (usual content of 60-80% in hop essential oil), are the most important. The content of terpenes like myracene, α -humulene, β -caryophyllene, and β -farnesene, as well as their ratio, represent the distinction of hop variety (1). The bitter compounds (α - and β -acids) are shown in Table 1 (2,3).

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Table 1. α -Acids and β -acids of hop

α -Acids			β -Acids	
Alcyl side chain (R)	Name	Formula	Name	Formula
-COCH ₂ CH(CH ₃) ₂ isovaleryl	Humulone	C ₂₁ H ₃₀ O ₅	Lupulone	C ₂₆ H ₃₈ O ₄
-COCH(CH ₃) ₂ isobutyryl	Cohumulone	C ₂₀ H ₂₈ O ₅	Colupulone	C ₂₅ H ₃₆ O ₄
-COCH(CH ₃)CH ₂ CH ₃ 2-methylbutyryl	Adhumulone	C ₂₁ H ₃₀ O ₅	Adlupulone	C ₂₆ H ₃₈ O ₄
-COCH ₂ CH ₃ propionyl	Posthumulone	C ₁₉ H ₂₆ O ₅	Postlupulone	C ₂₄ H ₃₄ O ₄
-COCH ₂ CH ₂ CH(CH ₃) ₂ 4-methylpentanyl	Prehumulone	C ₂₂ H ₃₂ O ₅	Prelupulone	C ₂₇ H ₄₀ O ₄

The difference in content of bitter compounds is characteristic for different hop varieties. Depending on α -acids content there are four categories of hop: fine aromatic hop (up to 5%), aromatic hop (5-6%), aromatic-bitter hop (6-8%), and bitter hop (8% and more of α -acids) (4). These compounds may be degraded or evaporated during storage, especially if storage conditions are not suitable. Using supercritical carbon dioxide (SC-CO₂) hop extracts instead of raw or palletized hop, many industrial problems (i.e. energy saving, no cooling demands, no storage problems, extract is stable at room temperature, etc.) could be prevented (5).

For separation of aromatic and bitter fractions of hop, supercritical fluid extraction using carbon dioxide as extractant (SFE-CO₂) in two extraction steps can be used. The extract containing mainly essential oil compounds could be obtained with CO₂ of lower solubility power (such as extractant at pressure of 150 bar and temperature of 40°C, i.e. solvent density of 0.790 g/cm³), and after that, second additional extract containing bitter hop compounds could be obtained by increasing extractant solubility power (adjusting operational conditions at pressure at 300 bar, and remaining constant temperature of 40°C, i.e. solvent density of 0.915 g/cm³).

For the purposes of mathematical modeling there are numerous models dealing with SFE of essential oils and related products from plant material. Reverchon (6) classified existing models as: 1. Empirical, based on Langmuir-like empirical equation, 2. Models originated from heat transfer analogy, where SFE is treated as heat transfer phenomena, and 3. Models based on differential mass balance, where mass balance along a section of

the extractor has been widely used to describe the behavior of fixed beds during solid/liquid operations like adsorption/desorption, reaction, and extraction.

Hortaçsu (7) classified available models as models where extraction is defined as chemical reaction, and models where extraction is considered as a physical phenomenon. The other approaches are Differential Mass Balance Models (i.e. Shrinking Core Model, Desorption-Dissolution-Diffusion Model) and other representations (like Models with Cellular Structure Representation, and Empirical Desorption Models).

EXPERIMENTAL

Material and methods

Plant material. Hop of Magnum cultivar was produced by the Institute of Hop, Broomcorn and Medical Plants, Bački Petrovac, Serbia and Montenegro (year 1999). Sample was dried cones of hop. Before extraction sample was milled, and mean particle diameter of 0.448×10^{-3} m was determined by sieve analysis.

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

Sample preparation. The procedure of obtaining samples was:

1. Yield of total extract (TE) was obtained by *n*-hexane using Soxhlet apparatus.
2. Extracts obtained by SFE-CO₂: Investigated hop sample (50.0 g) was extracted by carbon dioxide at temperature of 40°C and CO₂ flow rate of 97.725 L/h, in two steps, at 150 bar (Extract A) and 300 bar (Extract B), respectively. Extraction time for each step was 2.5 h. Separation conditions: $p = 15 \pm 1$ bar and $T = 20 \pm 1^\circ\text{C}$.

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

GC-MS Analysis. The GC instrument was a GCD HP G 1800 A (Hewlett-Packard, Palo Alto, Calif, USA). A column HP-5 MS (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 set initially at 50°C and was increased linearly at 20°C per minute to 130°C (1 minute) and then was increased by 9°C per minute until the final temperature of 280°C (8.33 min.). Total analysis time was 30 min. The injected volume of sample solution in methylenechloride - diethylether mixture in ratio 7 : 3 (10 mg/mL) was 5 μL (splitless injection). The mass spectrum was obtained using the SCAN-technique in 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 conductometric titration method modified by Wöllmer (10).

Modeling

For the purposes of the process mathematical modeling, several simple models were chosen. The first one is an empirical model in the form of Langmuir-like desorption isotherm (7,11,12):

$$Y = Y_{\infty} \frac{t}{B + t} \quad [1]$$

where Y and Y_{∞} are the extraction yields at any time t during extraction, and at infinite extraction time, respectively. B is a fitting model parameter. It represents all the characteristics of the natural material studied as well as the chosen extraction process. This model represents natural matrices extraction without any explicit concern for the actual process mechanism, but it still has advantage that it is simple and can be used for interpolation.

The second model is based on mass balance over an element of extractor, which is height dh . It was proposed by Reverchon (13) for the SFE of basil oil and it was applied with good accuracy, in original and form modified for fitting purposes, for SFE of thyme oil (9). With the hypothesis that the solvent density and flow rate are constant, Reverchon model equations are:

$$uV \frac{\partial c}{\partial h} + \varepsilon V \frac{\partial c}{\partial t} + (1 - \varepsilon) V \frac{\partial \bar{c}}{\partial t} = 0 \quad [2]$$

$$(1 - \varepsilon) V \frac{\partial \bar{c}}{\partial t} = -A_p K (\bar{c} - \bar{c}^*) \quad [3]$$

where ε is the bed porosity; V is the extractor volume; c is the extract concentration in the fluid phase (kg/m^3); \bar{c} is the extract concentration in the solid phase (kg/m^3); u is the solvent velocity (m/s); A_p is the particle surface (m^2); K is the mass transfer coefficient (m/s); \bar{c}^* is the concentration at the solid-fluid interface (kg/m^3); h is the bed height (m); t is the time (s).

The following assumptions were introduced: extraction is uniform all along the bed, external diffusion coefficients can be neglected, the term $A_p K / (1 - \varepsilon) V$ is constant, dimensionally equal to $1/\text{s}$, and therefore can be substituted with characteristic time $1/t_i$, where t_i is the internal diffusion time. Unknown concentration on the solid-fluid interface was eliminated with phase equilibrium relationship, and the model equations were further simplified by neglecting the accumulation of the extract in the fluid phase. Solving equations with appropriate initial condition, and introducing the normalized extraction yield, defined as:

$$Y = \frac{\bar{c}_0 - \bar{c}}{\bar{c}_0} \times 100 \quad [4]$$

where \bar{c}_0 is an initial concentration of extract in the solid phase (kg/m^3), the following equation was obtained:

$$Y = 100 \left[1 - \exp \left(\frac{-k_p t}{\frac{(1-\varepsilon) V \rho}{W} + k_p t_i} \right) \right] \quad [5]$$

where W is solvent mass flow rate (kg/s), ρ is the solvent density (kg/m³) and k_p is the volumetric partition coefficient of the extract between solid and fluid phase at equilibrium.

If the internal diffusion is the only limitation for mass transfer, and k_p is close to unity, the term $k_p t_i$ is by some orders of magnitude higher than the other one in the denominator of equation [5], and the equation can be further simplified to the form:

$$Y = 100 \left[1 - \exp \left(-\frac{t}{t_i} \right) \right] \quad [6]$$

Villiermaux (14) showed the relation between the diffusion time t_i and the diffusion coefficient D for different particle geometries

$$t_i = \mu \frac{l^2}{D} \quad [7]$$

where, for spherical particles, μ is equal to 3/5 and the characteristic dimension l (particle volume/particle surface) is $r/3$, where r is the mean particle radius.

The model can be fitted to experimental results with D as the only adjustable parameter. Schlieper (15) proposed a model as the characteristic time model, and introduced effective diffusion coefficient (D_{eff}) instead of diffusion coefficient (D). There was also an effective attempt (8) of determining D with the correlation of Ponomarev (16). For the fitting procedure, equation [6] can be modified to the form:

$$Y = 100 \cdot (1 - \exp(\alpha \cdot t)) \quad [8]$$

where $\alpha = -1/t_i$, and to the form

$$Y = 100 \cdot (1 - \exp(a \cdot t + b)) \quad [9]$$

where $a \cdot t + b = -t/t_i$. This modification was introduced by Zeković (9).

The single sphere model (7,8,12), based on heat transfer analogy is given with equation [10]. It is based on the same hypothesis for the macroscopic balances, defined in the characteristic time model:

$$Y = 1 - \frac{6}{\pi^2} \sum_{n=1}^{\infty} \frac{1}{n^2} \exp \left(-\frac{D_{eff} t n^2 \pi^2}{R^2} \right) \quad [10]$$

n being an integer. Model equation can be defined in a manner of fitting procedure, where D_{eff} can be obtained (12), or in a simple summation manner with defined integer number, and adopted D_{eff} value, previously estimated. The second approach has been used for SFE-CO₂-thyme modeling (9).

Since hop SFE has been performed as a two-step procedure under different experimental conditions, data for different operation pressures were modeled separately, i.e. modeling results for extracts A and B are given separately.

RESULTS AND DISCUSSION

Total extract (TE) yield was determined by *n*-hexane, using Soxhlet method of extraction. In order to prevent thermal decomposition of hop compounds, a temperature of 40°C was selected for SFE-CO₂. For obtaining extracts containing aroma and bitterness hop compounds two steps of SFE-CO₂ were used: SFE-CO₂ extraction at 150 bar, i.e. solvent density of 0.790 g/cm³, for 2.5 h (Extract A) and, after that, the same sample of hop Magnum was extracted at 300 bar, i.e. solvent density of 0.915 g/cm³ for 2.5 h (In this way Extract B was obtained). Results are shown in Table 2.

The content of α -acids in Extract B, as well as in native investigated hop, was determined using method of conductometric titration (Table 2).

SFE-CO₂ extraction kinetics of Magnum hop is presented in Figure 1.

GC-MS method was used for qualitative and quantitative determination of compounds contained in Extract A obtained in the first step of SFE-CO₂ (150 bar, 40°C and 2.5 h) process. Results of compound content in this extract are shown in Table 3.

Table 2. Results of Magnum hop extraction

Parameter	TE yield (%; g/100 g)	CO ₂ -extract yield at 150 bar (%; g/100 g)*	CO ₂ -extract yield at 300 bar (%; g/100g)**	α -Acids content (%) in:	
				Native hop	Extract B
Result	34.7	13.35	7.54	14.7	41.0

* Extract A; ** Extract B

Table 3. GC-MS results of Magnum hop extract A

Retention time t_R (min)	Compound	Compound content (%)
8.88	β -Caryophyllene	2.21
9.33	α -Humulene	10.35
18.83	Isohumulone	12.15
19.76	Colupulone	25.31
20.37	Adlupulone	6.93
20.45	Lupulone	36.37

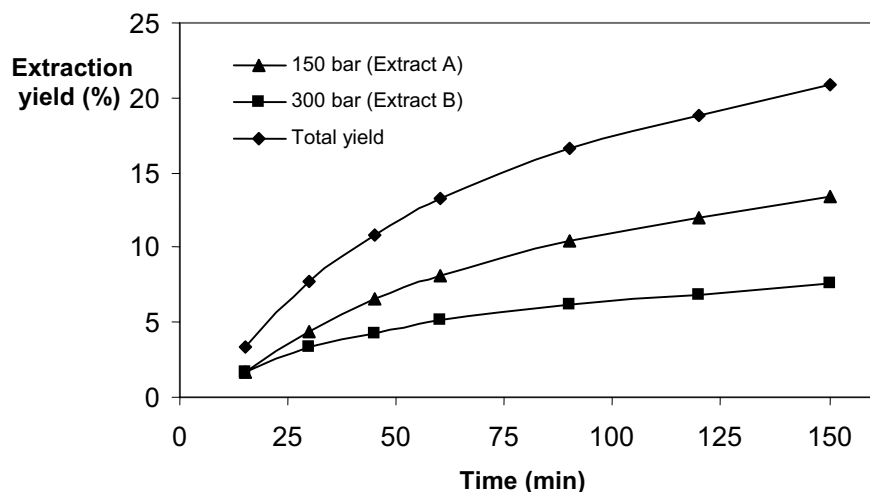


Fig. 1. Extraction kinetic of Magnum by SFE-CO₂

For the modeling purposes normalized extraction yield was defined as the ratio of extracted amount under defined operational condition, and initial amount of extractable substances present in plant matrices, estimated with classic *n*-hexane extraction process. For the Magnum hop variety initial amount of extractable substances was 34.70g/100g. The extraction processes for obtaining Extract A and Extract B were modeled separately.

The experimental results were fitted with:

- Empirical model (equation [1]), where B was a fitting parameter; Y_{∞} was determined as maximal extraction yield under defined operation conditions, i.e. it was 38.47 g/100g for $p = 150$ bar, and 35.31 g/100g for $p = 300$ bar.
- Characteristic time model equations [8], and [9] with α , a, and b as a fitted parameters. From the calculated α values, t_i were calculated, upon which effective diffusivities (D_{eff}) were calculated from equation [7].
- Single sphere model (equation [10]), with D_{eff} adopted from characteristic time model. Summation has been performed until the difference between two sum values has become less than defined precision (e.g. 10^{-4} was adopted for all calculations).

Calculations were performed in Origin 5.0 (empirical, characteristic time model), and MathCad for single sphere model. Precision was set to 10^{-4} . Modeling results are shown in Figures 2 and 3. The standard regression error (SRE) for all models was calculated using:

$$SRE = \sqrt{\frac{\sum (\text{experimental} \cdot \text{yield} - \text{calculated} \cdot \text{yield})^2}{N_{exp} - k}}$$

where N_{exp} is the number of experimental data points for each extraction curve, and k is the number of model parameters. The results are presented in Table 4.

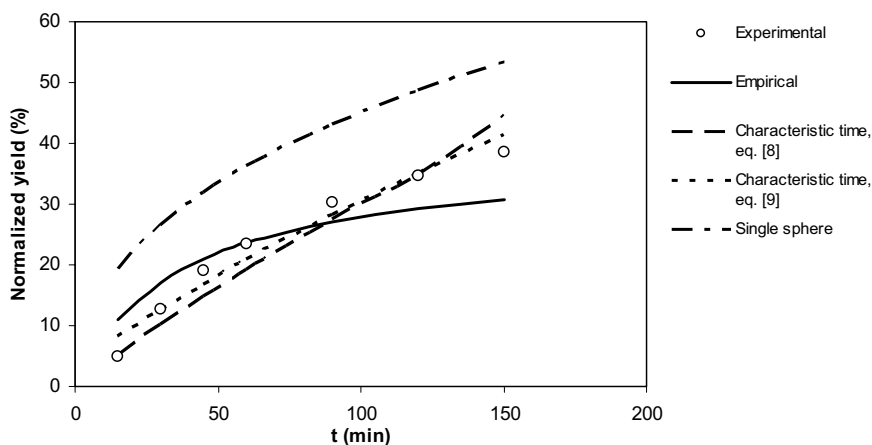


Fig. 2. Modeling results for SFE-CO₂ of Magnum hop Extract A

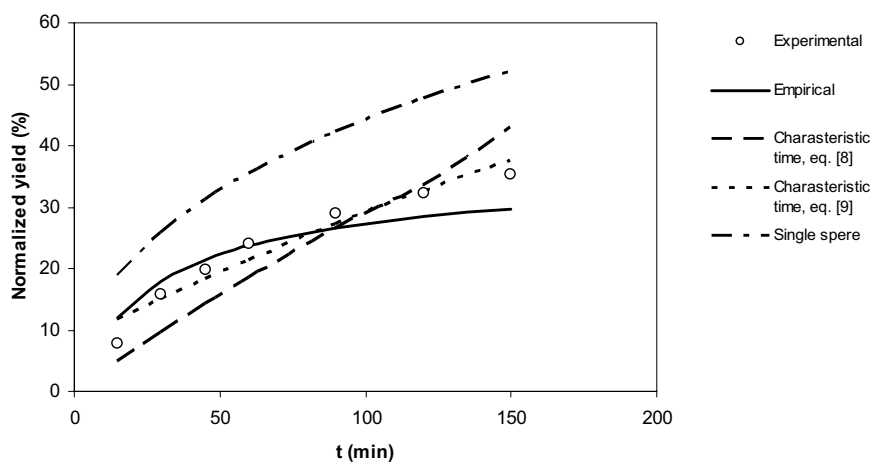


Fig. 3. Modeling results for SFE-CO₂ of Magnum hop Extract B

After separation of hop aroma fraction using SFE-CO₂ under extraction conditions of 150 bar, 40°C and 2.5 h (Extract A was obtained in this way), sample was extracted at higher solubility power of CO₂ (300 bar and same temperature of 40°C). In this way, after 2.5 hours of extraction, hop Extract B was obtained. The content of α -acids in native sample (14.7%) of Magnum cultivar, as well as in Extract B (41.0%) shows that Magnum is bitter category of hop.

Table 3 shows that the predominant compounds of Magnum hop Extract A are lupulone (36.37%), colupulone (25.31%), and isohumulone (12.15%).

Standard regression error (Table 4) showed that characteristic time model (Equation 9) best fitted experimental results, both for Extract A and Extract B. Empirical and characteristic time model (Equation 8) showed good agreement with experimental results. From

the parameter α in characteristic time model (eq. 8) effective diffusivities were obtained. Obtained lower diffusivity values ($1.9553 \times 10^{-13} \text{ m}^2/\text{s}$) for higher pressure values (Extract B), comparing to higher diffusivity values ($2.0500 \times 10^{-13} \text{ m}^2/\text{s}$) for lower pressure values (Extract A) can be explained by the fact that Extract B represents previously exhausted Extract A. Single sphere model poorly approximated experimental results, which is evident from Figures 2 and 3, as well as Table 4. The same poor results for single sphere model were obtained for SC-CO₂-thyme system. This indicates that this model can not be used in the access mode; instead, D_{eff} should be used as a fitting parameter.

Table 4. Parameters and standard regression error (SRE) for selected models

	Model parameters		SRE	
	Extract A	Extract B	Extract A	Extract B
Empirical	B = 37.6	B = 29.12	5.101	3.568
Characteristic time (eq. 8)	$\alpha = 5.972 \times 10^{-5}$	$\alpha = 5.697 \times 10^{-5}$	3.102	4.786
Characteristic time (eq. 9)	a = -0.00327 b = -0.0384	a = -0.00258 b = -0.0859	2.602	2.587
Single sphere	-	-	13.758	13.066

CONCLUSION

The SFE-CO₂ extract obtained for Magnum hop cultivar under operation conditions of 150 bar, 40°C (Extract A) during 2.5 h, contains only sesquiterpenes (i.e. humulene and caryophyllene) as hop aroma compounds. Since Extract A contains also certain amount of izohumulone, i.e. iso- α -acids, that influence hop bitterness, selected operation conditions did not provide complete separation of aromatic and bitter compounds. The relatively high amounts of three β -acids compounds (lupulone, colupulone, and adlupulone) were detected in Extract A. Magnum hop cultivar gave the quality extract (Extract A) with high amount of essential oil compounds.

The SFE-CO₂ extracts obtained at 300 bar, 40°C (Extract B) during 2.5 h, represents α -acids concentrates. Cultivars containing higher amounts of α -acids, as Magnum hop, give better extracts.

Mathematical modeling using empirical model, characteristic time model, and simple single sphere model has been performed on Magnum cultivar extraction experimental results, obtained at two pressures, i.e. in two series. Characteristic time model equations (modified and original) best fitted both series A (i.e. Extract A) and B (i.e. Extract B) experimental results. Empirical model showed good agreement, while single sphere model poorly approximated the experiments.

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ЕКСТРАКЦИЈА ХМЕЉА СУПЕРКРИТИЧНИМ УГЉЕНДИОКСИДОМ

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Испитана је екстракција хмеља сорте Магнум суперкритичним угљендиоксидом (SFE-CO₂) као екстрагенсом. Екстракција је изведена у два корака. Први корак представља екстракцију на 150 bar и 40°C у току 2,5 часа (екстракт А), а други екстракцију истог узорка на 300 bar и 40°C у току 2,5 часа (екстракт Б). Испитана је кинетика екстракције система хмељ - SFE-CO₂. У екстракту А детектована су два (α -хумулен и β -кариофилен) од четири уобичајена једињења која одређују арому хмеља. Поред њих, детектоване су изомеризоване α -киселине и β -киселине. У екстракту Б, садржај α -киселина је био висок (што указује на горку сорту хмеља). Експериментални резултати, који се односе на екстракцију хмеља сорте Магнум применом SFE-CO₂, математички су моделовани коришћењем емпиријског модела, модела карактеристичног времена, као и модела јединичне сфере. Модел карактеристичног времена најбоље је фитовао експерименталне резултате. Емпиријски модел је добро фитовао, док је модел јединичне сфере лоше апроксимирао експерименталне резултате.

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