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# Fractionation of hops-extract–ethanol solutions using dense $CO_2$ with a counter-current extraction column

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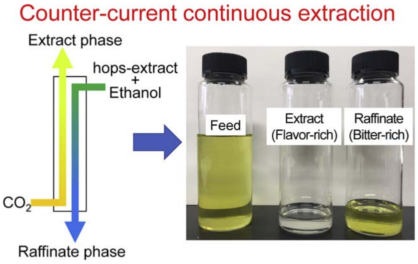
## GRAPHICAL ABSTRACT



## ARTICLE INFO

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## ABSTRACT

Hops-extract–ethanol solutions were contacted counter-currently with dense $CO_2$ in a 0.22 m column at temperatures of (313.2–353.2) K, pressures of (5.0–12.0) MPa, at solute-free solvent-to-feed (S/F) mass ratios of 3.8 and 8.6 for fractionating flavor and bitter compounds. Flavor-purity ratio ($P_f$) defined as flavor-compound mass over identified-compound mass was about 1.0 at 313.2 K, 6 MPa at S/F = 8.6. At these conditions, flavor-recovery ratio ($R_f$) defined as flavor-compound mass over feed flavor-compound mass was less than 0.03. At 353.2 K, 12.0 MPa at S/F = 8.6, maximum column productivity ($P_f = 0.76$, $R_f = 0.69$) was obtained. Conditions giving maximum purity and highest selectivity ($P_f = 0.99$, $R_f = 0.27$) were 333.2 K, 8.0 MPa at S/F = 8.6. In fractionation of feed solutions containing ethanol, column performance is dominated by ethanol distribution that is conveniently manipulated with conditions of the $CO_2$.

## 1. Introduction

The hops plant, *Humulus lupulus*, contains many flavors, resinous compounds and essential oils [1]. In the manufacture of beer, the addition of a small amount of hops is used for enhancing the taste and aroma of a beverage. Typically, hops plants are used commercially in the form of hops-extracts that are obtained by organic solvent extraction (e.g. ethanol and water) or supercritical fluid extraction (SFE), for which operating conditions are chosen to obtain high yields of the desired compounds. The use of SFE is widely studied for separation of complex natural-materials [2–7] because of the technique has merits regarding its use in food products and the lack of residue in the final product.

Compounds in hops-extract can be roughly divided into two groups: flavor and bitter compounds. Since the ratio of these ingredients greatly influences the final product, further separation techniques are required in hops-extract applications, such as high-aroma or strong-bitter tastes. Among the literature reporting on hops-extraction [8–17], Kupski et al. performed supercritical $CO_2$ extraction of hop pellets at different temperature and pressure conditions [8] and made an optimal

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mathematical model that correlated extraction behavior. Alejandro et al. investigated separation of one of the bitter compounds, xanthohumol, from hops-extract using supercritical fluid chromatography [9]. Formato et al. demonstrated supercritical carbon dioxide with co-solvent of ethanol could extract both alpha- and beta-acids from hop pellets [12]. Ethanol is an acceptable solvent for use in processing foodstuffs and its application is considered in this work.

The conventional separation methods for hops-extract usually use a semi-batch system and dried-crushed hops. To improve productivity of hops processing, a continuous counter-current extraction would be preferable for large-scale operation [18–29]. Although counter-current process is similar to a mixer-settler type of liquid–liquid extraction in terms of equilibrium distribution coefficients of the solute, the transport phenomena of the devices can be different. Namely, the mass transfer of the counter-current process tends to be enhanced through vapor-liquid contact over that of liquid–liquid contactors, so that the size of the installations can be greatly reduced. Brunner and Machado designed a process for the fractionation of fatty acids from palm fatty acid distillates with counter-current packed columns using supercritical $CO_2$ as solvent [24]. Fernandez-Ronco et al. investigated separation of oleoresin capsicum using supercritical $CO_2$ in a counter-current column based on equilibrium data [25]. Both of these processes were limited to $CO_2$-solute binary systems.

Reports on counter-current contactors with dense $CO_2$ adding polar alcohol aqueous solutions or water are available in the literature [30,31]. Fractionation studies that show recent applications of supercritical or dense $CO_2$ to fruit aroma and perfumes are given in refs [32,33] and a comprehensive review on fractionation technologies that shows application to beverages, biofuels, edible oils, essential oils, petroleum pitches, waxes and other substrates is available [34]. However, assessment of counter-current columns that use hops-extract substrates for the purpose of fractionation have not been reported. In previous work, distribution coefficients (*K*-values) of hops-extract–ethanol solutions with $CO_2$ were measured experimentally using a flow-type mixer-settler apparatus at temperatures ranging from 323.2 to 343.2 K, pressures from 8 to 10 MPa and feed concentrations of hops-extract in ethanol solvent from 0.5 to 5 wt% [35]. Considering the *K*-values from the obtained results, when a counter-current extraction with dense $CO_2$ is applied to a hops-extract–ethanol feed solution, flavor compounds that have low boiling temperatures will distribute to the $CO_2$-rich extract phase and bitter compounds that have high boiling temperatures will distribute to the ethanol-rich raffinate phase so that the desired fractions can be controlled with column conditions.

In this work, counter-current fractionations using pure $CO_2$ as extraction solvent (S) with feed (F) solutions consisting of hops-extract dissolved in ethanol were investigated at temperatures ranging from 313.2 to 353.2 K, pressures ranging from 5.0 to 12.0 MPa and solute-free solvent-to-feed (S/F) mass ratios of 3.8 and 8.6. Compounds contained in the hops-extract were separated and the effect of temperature, pressure and S/F ratio on flavor compound purity, recovery and selectivity were examined.

## 2. Experimental methods

### 2.1. Materials and chemicals

Carbon dioxide (99.5%) was obtained from Taiyo Nippon Sanso (Sendai). Ethanol (99.5%) was obtained from Wako Pure Chemical Industries (Osaka); β-myrcene (95.0%) and humulene (96.0%) were obtained from Sigma-Aldrich Co. Ethyl pentadecanoate (97.0%) used as internal standard in the GC analyses was obtained from Tokyo Chemical Industry Co., Ltd. Resin standards were obtained from American Society of Brewing Chemists Co., Ltd. Hops-extracts were obtained from a local supplier. Details of the hops-extract analysis are given in supplementary material (Figs. S1-S2). All chemicals were used as received.

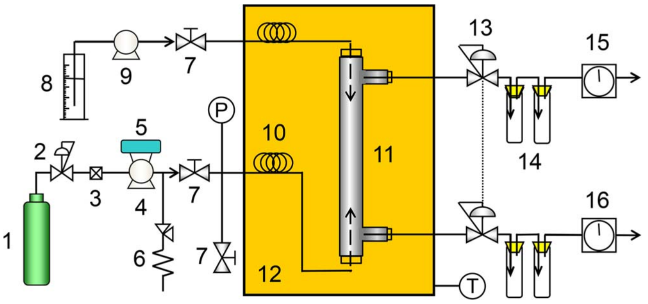

**Fig. 1.** Experimental apparatus for high-pressure counter-current extraction with $CO_2$ solvent as upstream solvent and hops-extract-ethanol solution as the downstream feed. 1. $CO_2$ cylinder, 2. Regulator, 3. Filter, 4. Pump, 5. Chiller, 6. Safety valve, 7. Valves, 8. Feed solution, 9. HPLC pump, 10. Preheating coil, 11. Packed extraction column, 12. Oven, 13. Back pressure regulator, 14. Traps, 15. Dry gas flow meter, 16. Wet gas flow meter.

### 2.2. Experimental apparatus and procedure

The counter-current flow apparatus is shown in Fig. 1. The apparatus consisted of separate delivery systems for the hops-extract–ethanol feed solution and the $CO_2$ solvent, respectively. An HPLC pump coupled with a cooling unit (Jasco Co., PU-2080-$CO_2$) was used to deliver $CO_2$ to the extraction column with $CO_2$ entering from the bottom of the column. The counter-current extraction column had a 6.23 mm internal diameter and was 218 mm in height that was filled with stainless steel 1.5 mm Dixon rings ($a_s = 3550\,m^2/m^3$) to promote phase contact. The void fraction of the packed-column was determined to be 87.5% by volumetric measurement. The $CO_2$ flow rate was quantified with a dry gas flow meter (Shinagawa Co. Ltd., DC-2). After $CO_2$ flow rate was controlled to a given value, pressure in the extraction column was increased gradually to the desired pressure. The supplied $CO_2$ passed through a preheating coil in which temperature was controlled by an oven (Shimadzu Co., CTO-20AC). The temperature of oven containing the extraction column was controlled to within an uncertainty of ± 0.5 K. Pressure was measured with a sensor (General Electric Company, PTX621) to within an uncertainty of ± 28 kPa. The feed solution of hops-extract–ethanol was supplied with a second HPLC pump (Jasco Co., PU-2085) to the top of the column. The hops-extract − ethanol solution concentration was varied in the range of 0.25–2 wt% in the measurements. The liquid feed flow rate was quantified volumetrically. The total mass flow rate range was 1.11–5.55 g/min. The $CO_2$ and the feed flow rates were varied in the range of (0.88–4.44) g/min and (0.230–1.11) g/min, respectively. The total mass flow rate was varied in the range of steady-state conditions and checked by confirming the constant flow rate of the materials. The $CO_2$ velocity calculated from volumetric $CO_2$ flow rate divided by the cross-sectional area of extraction column (void fraction $\varepsilon = 0.875$) were varied in the range of ($5.47 \times 10^{-3}$ to $1.28 \times 10^{-2}$) m/s.

Both extract and raffinate were collected for 30 min each in sampling traps cooled with acetone-dry ice mixtures after being depressurized by two coupled-backpressure regulators (Jasco Co., BP-2080-D). Liquid samples from the extract and raffinate phases were collected in traps and quantified with an electronic balance (Mettler-Toledo International Inc., AX-504). The gas contained in the extract phase was quantified with a dry gas flow meter and the gas contained in the raffinate phase was quantified with a wet gas flow meter (Shinagawa Co. Ltd., W-NK-0.5). Reproducibility was checked at 353 K, 8 MPa and S/F ratio of 8.6.

### 2.3. Analysis of hops-extract

The obtained flavor compounds of both extract and raffinate phases were analyzed using a gas chromatograph having a flame ionization detector (GC-FID, Agilent Technologies, Japan Ltd., GC-7890). The