Supercritical CO_2 Extraction of Terpenoids and Alpha/Beta-Acids from Canadian Cascade Hops

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1. Introduction

Hop is an important ingredient in the production of beer. Aroma and bitterness compounds are main components of hop with special effect on the beer taste. These compounds are mainly hydrocarbons, ketones, aldehydes, esters, carboxylic acids, alcohols, oxygen heterocyclic and sulfur compounds. The most important compounds are hydrocarbons, like terpenes, which usually contains 40-80% in hop essential oil[1]. Myrcene, α-humulene, β-caryophyllene and β-farnesene are the main components of terpenes with different amounts and ratios depending on the variety[2]. Also, bitter compounds (α and β-acids) are cohumulone, adhumulone, colupulone and adlupulone.

Cascade, one of the oldest and most used varieties for breweries in America, has essential oil composed of myrcene, humulene and caryophyllene, which contributes to the aroma of beer. The common methods of extraction of essential oils are solvent extraction with heptane (a toxic solvent that requires further separation) or steam distillation that uses high temperature affecting the quality of the final product. An alternative method, supercritical fluid extraction with carbon dioxide as the solvent is considered an environmentally friendly "green" extraction process. Supercritical carbon dioxide (SC-CO_2) technology has been used for the extraction of bioactives, alkaloids, essential oils and flavours[3]. SC-CO_2 is the ideal solvent for processing as it is non-toxic, non-flammable, cheap, and suitable for heat sensitive products, with good solvating power, and high diffusivity. Supercritical fluid technology is considered a green method to obtain natural compounds because the solvent is easily removed from the final product[3]. In addition, the composition of the extract is also altered by slight changes in pressure and temperature[3]. Thus, various parameters should be studied to obtain a high amount of target compounds[4]. No studies on Canadian hops extraction using SC-CO_2 are available. The objective of this study is to obtain bioactive compounds such as humulene, caryophyllene, and myrcene and beta-acids from hops using SC-CO_2 technology.

2. Materials and Methods

2.1. Materials

Cascade hops were kindly provided by Aratinga Inc. (Calgary, AB, Canada) and stored at -20 ºC for further usage. The sample was milled in a coffee grinder to reach the particle size of below 1mm and stored at 4 ºC. Solvents for the SC-CO_2 extraction were alcohol, water from the Milli-Q system (18.2 MΩ.cm, Millipore, Billerica, MA, USA) and CO_2 (99.9% purity) from Praxair (Edmonton, AB, Canada).

2.2. SC-CO_2 extraction

Extractions using SC-CO_2 were performed following procedures established in our laboratory at different pressures and temperatures[3]. Milled hops (2 g) was used to study the process conditions for maximum removal of terpenoids, and alpha and beta acids using the SC-CO2 extraction system (ISCO SFX 220, Lincoln, NE, USA). First, the sample was weighed and loaded inside the extraction cell, which had filters at the bottom and top. Then, the cell was inserted into the extraction chamber. The cooler was turned on to reach 0°C so that CO_2 is in liquid to be pumped. The one-way valve was opened between CO_2 tank and the extraction chamber. The working temperature was set on the extractor. Then, the desired pressure and time were set. After reaching the desired temperature and pressure, the dynamic extraction started. The extraction was performed at different pressures of 100, 200 and 300 bar and temperatures of 40 and 60°C with 8 mL/min of CO_2 flow rate (at pump condition). All extractions were performed in duplicate and total hops extract was collected every 10 min in glass tubes placed in an ice bath. The tubes with the hop extracts were kept at room temperature (22°C) for 20 min prior to the gravimetric quantification. Then, extracts were analyzed for humulene, caryophyllene, and myrcene contents as well as for alpha- and beta-acids.

3. Results and discussion

Fig. 1 shows the effect of temperature (40 and 60 ºC) and pressure (100-300 bar) on the yield of the extract after SC-CO_2 extraction. The maximum yield (13.9%) was obtained at 300 bar and 40ºC/60ºC for 30 min. Using SC-CO_2, the majority of extract was removed in the first 10 min of extraction (solubility effect), but no significant increase ($p > 0.05$) was observed after 20 and 30 min (mass transfer predominance) at all conditions. The amount of extract increased significantly ($p < 0.05$) from 10 to 30 min at 100 bar/40 °C from 9.22 to 11.52%. The yield of extract significantly ($p < 0.05$) influenced by temperature and pressure. The total extract yield increased from 9.22%, to 11.38% and 12.66% at 100, 200 and 300 bar, respectively at a constant temperature of 40°C. Also, after 20 and 30 min of extraction, there was a significant increase in the extract yield from 10.64 to 13.30% and 11.52 to 13.85% at 100 and 300 bar.

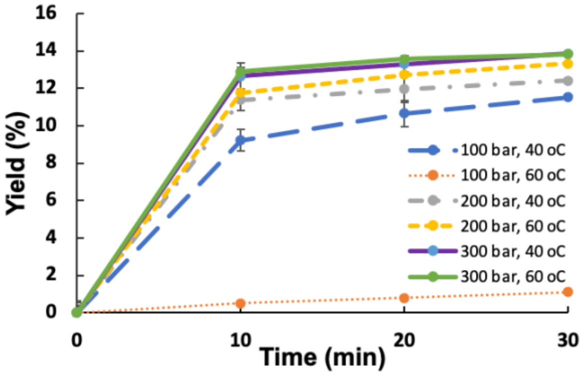

Fig. 1. Yield obtained after SC-CO_2 extraction at different pressure and temperature conditions.

Fig. 2 shows yields of beta-acids (colupulone and adlupulone) obtained after SC-CO_2 extraction at different pressure and temperature conditions. The maximum yields were obtained for colupulone and adlupulone at 100 bar and 40ºC for 30 min.

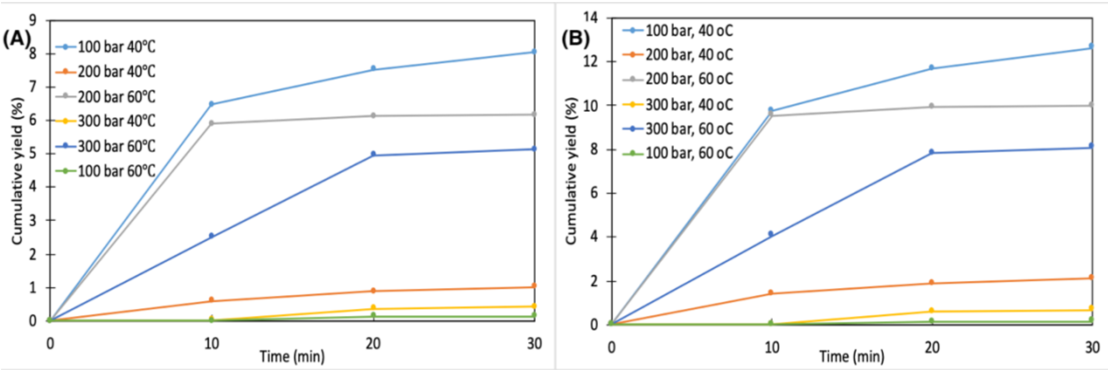

Fig. 2. Yields of beta-acids (A) colupulone and (B) adlupulone obtained after SC-CO_2 extraction at different pressure and temperature conditions.

4. Conclusions

The main compounds extracted from Cascade hops using SC-CO_2 were humulene, caryophyllene and myrcene. The increase of pressure from 100 bar to 200 bar led to an increase of terpenoids contents using SC-CO_2 extraction, with a maximum removal at 40ºC and 200 bar for 30 min. The use of SC-CO_2 enhanced extractability of terpenoids and beta-acids. Also, higher extraction yields were obtained for acids using SC-CO_2 compared to the hydrodistillation process.

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