Analysis of Supercritical Carbon Dioxide Extracts from Cones and Leaves of a *Humulus lupulus* L Cultivar

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ABSTRACT

Extracts obtained by supercritical carbon dioxide extraction of cones and leaves of hop (*Humulus lupulus* L) at different combinations of temperature and pressure were analysed for their α- and β-acids and volatiles, using HPLC and GC respectively. The yield and composition of the mixtures of bitter compounds from the cones were largely influenced by the temperature and pressure applied during the extraction. Bitter compounds could not be detected in the extracts from the leaves. The most important volatile components identified were β-myrcene, β-caryophyllene and α-humulene. The extraction parameters also influenced the composition of the mixture of volatiles from the cones and leaves, 40°C and 20.0 MPa being optimum for the extraction of both bitter compounds and volatiles, which are thought to be important for the aroma of beer.

Key words: Hop, *Humulus lupulus* L, supercritical fluid extraction, carbon dioxide, hop cones, hop leaves, essential oil, bitter acids, GC, GC-MS, 1H-NMR, HPLC.

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INTRODUCTION

Various cultivars of hop, *Humulus lupulus* L, are used in the brewing process to impart a bitter taste and hop flavour, as well as a characteristic aroma, to beer (Verhagen 1988). The glandular hairs (lupulin glands) of the hop cones contain a resinous fraction responsible for the bitter taste of beer and a volatile fraction which contributes to its flavour and aroma. Breweries are known to use hop cones or hop pellets and hop extracts. The extracts are produced either by means of organic solvents, such as methylene chloride, ethanol or hexane, or by use of liquid or supercritical carbon dioxide.

Extraction with liquid or supercritical carbon dioxide has been suggested as a viable alternative to other extraction techniques (Grimmet 1981; McHugh and Krukonis 1986; Vollbrecht 1987; Stahl et al 1988). The procedure is mainly a normal extraction, although it shows some aspects of distillation. The application of supercritical fluid extraction is based on the strong variation of the dissolving capacity of the solvent near its critical point. Mass transportation with supercritical fluids is fast, although the state of the fluid, established by pressure and temperature, shows a density value similar to those of liquid solvents; however, their dynamic viscosity ($\eta$) is nearer to that of the gaseous state and their diffusion coefficient ($D$) is, near the critical point, more than ten times that of a liquid (Gouw and Jentoft 1972). So, in principle, supercritical fluids penetrate more efficiently into the material, and, depending on the parameters, can extract the soluble components to a greater extent.

The main advantages of supercritical fluid extraction (SFE) with carbon dioxide are:

(a) the extraction takes place at comparatively low temperatures (31.1–100°C) which preserve the integrity of thermally labile components (Stahl and Schütz 1978, 1980);
(b) the energy consumption is small, compared with solvent extraction or steam distillation;
(c) carbon dioxide as such is not toxic, and it is easily removable from the extract.

The well established superiority of carbon dioxide extracts over those prepared using other solvents (Anon 1982; Moyler 1984; Vollbrecht 1987), the frequent use of carbon dioxide for extraction of hops on an industrial scale, and the fact that most of the industrially applied procedures are patented, prompted us to undertake the following investigations. The analysis of supercritical carbon dioxide extracts from cones and leaves of the hop cultivar Wye Northdown is described. The extracts obtained at different combinations of temperature and pressure were compared qualitatively as to their volatile and bitter compounds. Supercritical instead of liquid carbon dioxide was used, since the former has more different combinations of temperature and pressure (Grimmet 1981; Vollbrecht 1987).
MATERIALS AND METHODS

Plant material

_Humulus lupulus_ L cv Wye Northdown was grown in the pharmacognostical garden near the Gorlaeus Laboratories, Leiden University. The cultivar was supplied by the Land- en Tuinbouwschool, Vrij Technisch Instituut, Poperinge, Belgium. Cones and leaves of the plants were collected in the first half of September 1988 and dried at room temperature without air circulation to a moisture content of 10–15%.

A voucher specimen has been deposited in the Herbarium Vadense, Agricultural University of Wageningen, The Netherlands.

Supercritical carbon dioxide extraction

The cones and leaves of Wye Northdown were separately extracted with supercritical carbon dioxide in an extraction plant at the Laboratory for Process Equipment of the Delft University of Technology, Delft; a schematic diagram of the apparatus is given in Fig. 1. Gaseous carbon dioxide from the cylinder (1) and from the separator (8) is liquefied in the condenser (2) and subsequently collected in the storage vessel (3). After being compressed by the pump (4) and heated to the temperature selected (5), the supercritical carbon dioxide enters the 1-litre extraction vessel (6), where the pressure is controlled by a back-pressure regulator (7). By reducing the pressure to c 5 MPa, the extract is separated from the solvent (carbon dioxide) in the separator (8), since a decrease of temperature and pressure causes a decreased dissolving capacity. The extract can be collected during the process or afterwards via a valve in the bottom of the separator. The separator is heated in order to evaporate the carbon dioxide, which is subsequently recycled to the condenser (2).

A 40-g sample of cones or leaves was packed as tightly as possible in a stainless steel cylinder (0.6 litre), which was subsequently brought into the extraction vessel, which is also kept at the extraction temperature selected.

The extractions were carried out in duplicate at four combinations of pressure and temperature (Table 1). In each experiment, three fractions were collected in liquid carbon dioxide in the separator, one fraction each hour, except for experiment 2 (E 2), where six fractions were collected during 6 h; the fractions were finally dissolved in pentane. Special precautions were taken to clean the separator.
TABLE 1
Parameters used for supercritical carbon dioxide extraction of hop cones and leaves

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Plant material</th>
<th>Temperature (°C)</th>
<th>Pressure (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1</td>
<td>40 g cones</td>
<td>40</td>
<td>12.5</td>
</tr>
<tr>
<td>E2</td>
<td>40 g cones</td>
<td>40</td>
<td>20.0</td>
</tr>
<tr>
<td>E3</td>
<td>40 g cones</td>
<td>40</td>
<td>27.5</td>
</tr>
<tr>
<td>E4</td>
<td>40 g cones</td>
<td>60</td>
<td>20.0</td>
</tr>
<tr>
<td>E5</td>
<td>40 g leaves</td>
<td>40</td>
<td>20.0</td>
</tr>
</tbody>
</table>

Analysis of the fractions
Each fraction was divided into two parts; one was analysed by GC and GC–MS for volatiles and the second one by HPLC and ¹H-NMR spectroscopy for the bitter compounds.

GC and GC–MS analysis
GC analyses were carried out on a dual channel gas chromatograph, Packard model 439 (Chrompack b.v., Delft), equipped with FIDs and connected with two chromatographic data processors, Chromatopac C-R3A (Shimadzu Corp, Kyoto, Japan). GC conditions were as follows: columns: fused silica, 50 m x 0.23 mm id and 60 m x 0.25 mm id, coated with CP-Wax 52cb, film thickness 0.22 μm (Chrompack bv, Middelburg, The Netherlands), and Durabond DB-1, film thickness 0.25 μm (J & W Scientific Inc, Rancho Cordova, CA, USA), respectively; oven temperature: programmed, 45–240°C at 3°C min⁻¹, and subsequently isothermal at 240°C for 15 min; injector and detector: 220°C and 240°C respectively; carrier gas: nitrogen; split sampling technique: ratio 1 to 100; sample size: 1 μl of a fraction in n-pentane. The percentage composition of the samples was computed from the GC peak areas without using correction factors.

GC–MS data were obtained on a gas chromatograph, Packard model 438 A, equipped with a fused-silica column, 50 m x 0.22 mm id, coated with CP-Sil 5cb, film thickness 0.13 μm (Chrompack) and interfaced with a Finnigan MAT 700 ion trap detector (ITD; software version 3.0; Finnigan MAT, San Jose, CA, USA). Conditions were as follows: GC oven temperature: as above; transfer line: 250°C; carrier gas: helium, 150 kPa; split sampling technique: ratio 1 to 40; scan range: 40–249 u; scan time, 1 s.

HPLC analysis
HPLC analyses were carried out with an LKB 2150 pump, and a UV monitor LKB 2151 (Pharmacia, Woerden, The Netherlands) connected to a Kipp BD 40 recorder.
Supercritical carbon dioxide extracts from hop cones and leaves

(Kipp & Zn, Delft) and a Packard 603 integrator (Chrompack bv); for injection a Rheodyne 7125 injector (Pharmacia) was used. The HPLC conditions were as follows; column: Chromsep, 100 x 4.6 mm, Microsphere C18, particle size 3 μm (Chrompack bv); eluent: methanol/water/phosphoric acid [85%] (85:17:0.25), flow rate: 1.5 ml min⁻¹; detection: UV light at 314 nm; sample preparation: after the evaporation of pentane 100 mg of the extract was dissolved in 9.0 ml methanol and 1.0 ml internal standard (p-nitroanilide of myristic acid, 1.32 mg ml⁻¹ methanol); sample size: 10 μl.

H-NMR spectroscopy

H-NMR spectra of the extracts were recorded at 300 MHz in CDCl₃ with TMS as internal reference on a Bruker WM-300 spectrometer (Bruker Analytische Messtechnik GmbH, Rheinstetten/Karlsruhe, FRG), equipped with an Aspect 2000 data system. The values are given in δ-units.

RESULTS AND DISCUSSION

As the viscosity and density of supercritical carbon dioxide vary with temperature and pressure (Grimmet 1981), the compositions of the mixtures of bitter compounds and volatiles extracted from hop cones and leaves at the combinations of temperature and pressure given in Table 1 were studied.

A comparison of the extracts from the cones of Wye Northdown clearly showed that the highest yield was obtained in experiment E2. The extraction of 40 g of cones at 40°C and 20.0 MPa for 3 h yielded 4.3 g of extract (c 11%): 1.42 g, 1.85 g and 1.03 g for the fractions 1, 2 and 3 respectively. These extracts were yellow, translucent and devoid of chlorophyll, and had the smell of fresh hops. According to Stahl et al (1988) relatively high pressures (up to c 35 MPa) are needed to give good yields of hop resins.

The concentrations of the bitter compounds in the extracts were determined by HPLC as described by Verzele et al (1983), Verhagen (1988) and Buckee (1985). The presence of these bitter acids was confirmed by their characteristic hydroxy proton signals from δ 18.3 to 19.4 in the H-NMR spectra of the supercritical carbon dioxide extracts (De Keukeleire 1982).

In the experiments E1, E2 and E3, the temperature was kept constant at 40°C, whereas the pressures applied were 12.5, 20.0 and 27.5 MPa. In Fig 2 the concentrations of the α-acids (humulone, co- and adhumulone) and β-acids (lupulone, co- and adlupulone) in the fractions 1–3 of the experiments are shown. Extraction at 12.5 MPa (E1) or 27.5 MPa (E3) resulted in lower concentrations of α- and β-acids, especially in the fractions extracted during the first hour, which did not contain any β-acids. The total amounts of bitter compounds extracted during 3 h were also smaller than in experiment E2.

Since the extraction rate of the bitter compounds appeared to be constant during the whole period of extraction in experiment E2, this period was extended for a further 3 h. Although the amounts of extract decreased drastically during the
extended period (fractions 4–6), the concentrations of the α- and β-acids were rather constant: they declined only in fraction 6, to 18% and 4% respectively.

Comparison of the results of the experiments E2 and E4, in which the pressure was kept constant but the temperatures were 40°C and 60°C respectively, clearly showed that the β-acids in particular were present to a lesser extent in all three fractions; the concentration of the α-acids was considerably lower in the first fraction, but only slightly lower in fractions 2 and 3. No bitter compounds were detected in the leaf extracts of Wye Northdown.

The extracts obtained by supercritical carbon dioxide extraction under the conditions described above were also analysed for their volatile components. The volatiles identified were similar to those reported for the essential oils, obtained by hydrodistillation from the cones or the leaves of some hop cultivars (Katsiotis et al. 1989, 1990). The relative amounts of the most interesting volatile components of the extracts from the cones or leaves are depicted in Fig 3 as GC peak area percentages. These components were β-myrcene, β-caryophyllene, α-humulene, γ- and δ-cadinene, and β-caryophyllene epoxide. As the amounts of γ- and δ-cadinene were almost similar in all fractions, only the GC peak area percentage of one of them is given in Fig 3.

The temperature and pressure applied during the extractions influenced the composition of the mixtures of volatiles present in the fractions of the various extracts. Selection of extraction parameters may thus be used to obtain a desired composition. The conditions applied for experiment E2, which yielded the largest
Fig 3. Peak area percentages of some volatile components in supercritical carbon dioxide extracts from hop cones and leaves. F-1 to F-3, fractions of the extracts collected during the first, second and third hour of extraction, respectively.
amounts of extract, led to an α-humulene-rich mixture of volatiles. The highest concentrations of the volatiles identified were present in the fractions of experiment E2 (40°C; 20.0 MPa) which is in agreement with the results of Nykänen et al (1989). The largest amounts of β-myrcene were found in the first fractions, except in experiment E2. Figure 3 clearly shows that the amounts of sesquiterpenes extracted still increased when that of β-myrcene was already decreasing, and that in experiment E3 (40°C; 27.5 MPa) the extract obtained after 2 h was very deficient in the volatiles identified.

The almost complete absence of β-myrcene in the extracts from the leaves (experiment E5) was consistent with the data found in the analysis of the hydrodistilled oil from hop leaves (Katsiotis et al 1990). The same was true for β-caryophyllene being the main component in these extracts.

It can be concluded that the composition of the mixture of volatiles extracted by supercritical carbon dioxide is, to a large extent, dependent on the conditions of the extraction fluid. Experiment E2 afforded maximum yields of the extracts quantitatively. These extracts were richer in the bitter compounds and volatiles identified. Obviously, the extraction parameters of experiment E2 were optimum as compared with the other experiments carried out.

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Supercritical carbon dioxide extracts from hop cones and leaves