Preparation and Analysis of Liquid CO_2 Hop Extracts[1]

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ABSTRACT

Hop extracts were prepared from several hop varieties grown in Europe, America, and Australia, using liquid CO_2 as the extracting solvent. Significantly less of the soft resin fraction was extracted by liquid CO_2 than by either *n*-hexane or methylene chloride. However, approximately 95% of the α-acids and 95–100% of the β-acids were extracted by liquid CO_2 in all cases. The composition of the essential oil fractions isolated by vacuum steam distillation of the extract and the hops from which the extract was derived were found to be very similar. None of the hard resin fraction was extracted by liquid CO_2. Selected liquid CO_2 extracts were compared analytically with commercial solvent extracts prepared from the same hop variety.

Key words: *α-Acids, β-Acids, Hop extract, Hop oil, Liquid CO_2*

The potential of CO_2 as a solvent, whether in the liquid or the supercritical state, has been known for some time. In particular, the application of liquid CO_2 to the extraction of plant material, including hops, was investigated in some detail by Russian workers (8). However, in the last five years the use of liquid CO_2 for the extraction of hops has become of ever-increasing interest to brewers, hop growers, and extract manufacturers in Europe, the United States, Asia, and Australia.

This rapid development has been stimulated, in large part, by the general concern about solvent residues in foodstuffs. It has been aided by the fact that liquid CO_2 has been shown to produce a selective, high quality, stable hop extract. Although some doubts were expressed initially (3), the use of liquid CO_2 for hop extraction on a commercial scale is now both a practical and economic proposition. This acceptance of the commercial viability of liquid CO_2 as a solvent is a consequence of the initial development conducted jointly by the Brewing Research Foundation and The Distillers Company (CO₂) Ltd. in the United Kingdom (7). In 1977, Laws et al outlined the process and described the type of extracts that were obtained in a pilot plant from a number of hop varieties (6).

At about this time, stimulated by the results of the investigations at the Brewing Research Foundation and by the fact that the company was both a committed manufacturer and user of hop extract, Carlton and United Breweries Limited commenced investigations into the use of liquid CO_2 for extraction of Australian hops, specifically the variety "Pride of Ringwood," which constitutes virtually the whole of the Australian hop crop.

EXPERIMENTAL

Extraction Plant

Preliminary investigations were conducted on a laboratory scale (100 g of hops), using a batch extraction principle, but progressed fairly rapidly to a pilot plant scale. This scale-up was necessary to obtain the design data needed for the construction of a full-scale commercial plant.

The pilot plant consisted of seven insulated extraction columns, which could be used in series, in parallel, or in isolation, and an evaporator. Liquid CO_2 was supplied to the plant by CO_2 cylinders equipped with diptubes. The liquid CO_2 was cooled by passing the feed line through a glycol/water bath to control the temperature of the liquid entering the extraction columns.

To avoid vaporization of the CO_2 during passage through the extraction columns, the temperature of the liquid CO_2 entering the columns was cooled to below the equilibrium temperature corresponding to the pressure in the CO_2 supply cylinders. The evaporator was equipped with a hot water jacket to provide the heat necessary to evaporate the liquid CO_2. The CO_2 venting rate, and hence the liquid CO_2 flow rate through the system, was controlled by a needle valve in the exit line. The gaseous high pressure CO_2 stream from the evaporator passed through a vapor heater so that after expansion across the needle valve to atmospheric pressure, the exhaust gas was at approximately ambient temperature.

This plant was used for extraction of all the varieties referred to in this article. In addition, Pride of Ringwood hops from both the 1979 and 1980 crops were extracted in the commercial plant. Apart from the large scale-up factor involved between the pilot plant and the commercial plant (~ 600 times), the major difference between the two plants was the absence of a bulk liquid CO_2 supply and recycle facility in the pilot plant.

Hops

In all cases the hops submitted to extraction were obtained in the form of pellets from various commercial suppliers and were remilled through a hammer mill before being packed into the extraction column. Fuggle and Cascade pellets were enriched.

Extraction Conditions

The extraction conditions were held constant on the basis of what had been found satisfactory for extraction of Pride of Ringwood hops, accepting the fact that these conditions might not be optimum for extraction of all varieties tested.

Hop pellets were milled in a hammer mill, and the milled hops (normally 900 g) were packed by gravity into a single extraction column. Liquid CO_2 was then passed upwards through the hops at a flow rate of 20 kg/hr for 4 hr with an inlet temperature of $+7°C$ and an operating pressure of $\sim$ 5,500 kPa ($\sim$ 800 psi).

At the end of the extraction period, during which the liquid CO_2 was vented to atmosphere as described above, the extract was recovered by being drained from the bottom of the evaporator.

Analyses of hops extracted by this process and of hops just before extraction are detailed in Table I. Extracts of the Pride of Ringwood variety were prepared in both the pilot and the commercial liquid CO_2 extraction plants.

Determination of Lead Conductance Value

Hops. A representative portion of powdered hops (10 g) produced by hammer milling hop pellets was transferred to a stainless steel extraction cannister. Trichloroethylene (200 ml) and six steel balls were added, and the cannister was sealed and rotated on a motorized rack for 30 min. The resultant extract was filtered through glass wool, and an aliquot (5 ml) of the filtrate was diluted with methanol ($\sim$ 50 ml). The resultant solution was titrated conductometrically with standard methanolic lead acetate solution (0.1 N) and the lead conductance value (LCV) calculated.

Hop Extracts. A sample of hop extract ($\sim$ 2 g) was accurately weighed and dissolved/dispersed in methanol, and the volume was made to 100 ml with methanol. An aliquot (5 ml) of the methanol solution was titrated conductometrically with standard methanolic lead acetate solution (0.1 N) and the LCV calculated.

Determination of α-Acids and β-Acids by High Performance Liquid Chromatography

Hops. An extract of hops was prepared as described above, using methanol instead of trichloroethylene. An aliquot (5 ml) of the methanol extract of hops was diluted to volume (50 ml) with methanol. An aliquot of this methanolic solution was mixed with an internal standard solution and the resultant solution analyzed by high performance liquid chromatography (HPLC).

Hop Extracts. A methanolic solution of hop extract was prepared and an aliquot mixed with an internal standard solution as for hops. The resultant solution was used for chromatographic analysis.

Chromatography. Analysis of the samples was carried out using a reverse phase chromatographic procedure developed in the Company's Central Laboratories.[2]

Determination of n-Hexane Soluble Material

Hops. A representative sample (10 g) of hop powder produced by milling hop pellets was placed in an extraction thimble in a Soxhlet apparatus containing n-hexane ($\sim$ 300 ml) and extracted under reflux. Extraction was continued for approximately 4 hr (equivalent to approximately 20 batch extractions). The n-hexane was then removed in vacuo and the flask weighed to constant weight.

Hop Extracts. All liquid CO_2 extracts were completely soluble in n-hexane at ambient temperature (20°C).

A sample of a commercial solvent extract ($\sim$ 1 g) was accurately weighed, dissolved in methanol ($\sim$ 20 ml) and quantitatively transferred to a separating funnel (250 ml capacity). n-Hexane (100 ml) was added, followed by hydrochloric acid (3 M, 50 ml) and the funnel was shaken. After separation of the n-hexane layer, the aqueous layer was shaken with additional volumes of fresh n-hexane (2 × 100 ml). The n-hexane extracts were combined, washed with saturated aqueous sodium chloride solution, and dried over anhydrous sodium sulphate. The n-hexane was removed in vacuo and the flask weighed to constant weight.

Determination of Methylene Chloride Soluble Material

Hops. The procedure described for n-hexane solubles was followed, with the substitution of methylene chloride for n-hexane.

Hop Extracts. All hop extracts examined were completely soluble in methylene chloride at ambient temperature (20°C).

Determination of Total Essential Oil Content

Hops. A sample (100 g) of powdered hops was placed in a round-bottomed flask (3-L) with distilled water (1,500 ml). The mixture was distilled with cohobation for 2 hr, the essential oil layer being collected in an oil trap. At the end of the distillation period the volume of oil was measured.

Hop Extracts. A sample (20 g) of hop extract was accurately weighed into a round-bottomed flask (3-L), and distilled water (1,500 ml) was added. The mixture was distilled with cohobation in the same manner, and the volume of essential oil collected was measured.

Vacuum Steam Distillation of Essential Oil

An accurately weighed amount of milled hops ($\sim$ 10 g) or hop extract ($\sim$ 3 g) suspended in water (100 ml) in a round-bottomed flask (250-ml capacity) was frozen with liquid N_2. The flask was connected to a vacuum pump through two liquid traps cooled in

TABLE I
Analysis of Hops Extracted with Liquid CO_2[a]

Hop	Country	Year	α-Acid (% w/w)	β-Acid (% w/w)	LCV[b]
Hallertau Aroma	F.D.R.	1979	4.6	4.2	5.7
Hallertau Northern Brewer	F.D.R.	1979	7.3	3.3	8.5
U.K. Northern Brewer	U.K.	1979	6.8	3.3	7.6
Wye Challenger	U.K.	1979	6.8	4.1	7.5
Oregon Bullion	U.S.A.	1979	7.5	4.4	8.9
Oregon Fuggle (enriched)	U.S.A.	1979	6.5	5.7	8.1
Washington Cascade (enriched)	U.S.A.	1979	7.7	8.5	9.4
Yakima Cluster	U.S.A.	1979	6.3	4.5	7.4
Pride of Ringwood	Australia	1979	8.7	5.1	10.3
Pride of Ringwood	Australia	1980	9.0	4.9	10.2

[a] Analytical data calculated on an "as is" basis.
[b] Lead conductance value.

TABLE II
Comparative Solvent Properties for Hop Extraction

	Solvent Extractibles, % w/w[a]		
Hop Variety	Liquid CO_2	n-Hexane	Methylene Chloride
Hallertau Aroma	12.2	15.6	17.5
Hallertau Northern Brewer	15.8	18.3	22.3
U.K. Northern Brewer	17.3	19.6	24.1
Wye Challenger	18.1	22.5	27.4
Oregon Bullion	18.4	23.7	34.5
Oregon Fuggle (enriched)	17.4	22.1	33.1
Washington Cascade (enriched)	22.4	28.2	34.7
Yakima Cluster	13.2	17.4	22.6
Pride of Ringwood, 1979	18.1	23.6	31.7
Pride of Ringwood, 1980	20.2	23.3	28.6

[a] Calculated on an "as is" basis.

liquid N_2, and the system was evacuated to $2-5 \times 10^{-2}$ Torr (2.7-6.7 Pa) and then isolated from the pump. The flask was warmed, first with cold water (10°C) and then in a water bath at 40°C. The flask was maintained in a water bath at 40°C and the distillate was collected in the first receiver, which was cooled in liquid N_2. The total distillate ($\sim$ 80-90 ml for hops, distillation time $\sim$ 1.5 hours, or $\sim$ 100 ml for hop extract, distillation time 2 hr) was thawed and extracted with Freon 11™ (fluorotrichloromethane, 1-1.5 ml).

Gas Chromatography

The Freon extract was subjected to gas chromatography on an HP5880 gas chromatograph under the following conditions. The column was glass WCOT (37 m × 0.2 mm) coated with OV101 (grade C). Gases were: carrier helium, 0.6 ml/min; make up helium, 30 ml/min; hydrogen, 30 ml/min; air, 450 ml/min. Oven temperature was held at 65°C for 10 min, increased at 2°C/min to 160°C, and held at 160°C for 10 min. Injector temperature was 190°C. Detector temperature was 200°C. Sample size was 1 μl, split ratio 1:20, and attenuation 2^3.

RESULTS AND DISCUSSION

Resin Composition of Extracts

The selectivity of liquid CO_2 as an extracting solvent has been highlighted in the recent literature. This is one of the solvent's more interesting aspects in view of its effect on the composition of the final extract obtained. The significance of this factor is demonstrated in Table II, in which the proportion of liquid CO_2 solubles obtained in the pilot plant is compared with the proportion of extractable material obtained using, with Soxhlet extraction, n-hexane and methylene chloride, the two most commonly used commercial solvents. Liquid CO_2 extracts the least material of the three solvents. Only approximately 75-80% of the n-hexane solubles were extracted under the conditions described, except that from the Hallertau Northern Brewers, U.K. Northern Brewers, and 1980 Pride of Ringwood hops somewhat more, $\sim$ 87%, was extracted. Despite the incomplete recovery of n-hexane soluble material, recoveries of α-acids were always approximately 95% and of β-acids 95% or more (Table III), thus indicating the much greater selectivity of the liquid CO_2 under the extraction conditions used. Optimization of extraction conditions for particular varieties should result in extraction efficiencies of 95% or more for α-acids in all cases. In this respect, as has been indicated by Kruger (3), liquid CO_2 differs significantly from supercritical CO_2. With the latter solvent, the extraction is significantly less selective.

Whereas virtually all the α-acids and β-acids, as determined by HPLC, were extracted, the proportion of LCV material extracted

was, on the average, only about 80%, with the exception of that from the Northern Brewer and Wye Challenger varieties. This again highlights the selectivity of the solvent and indicates that in order to obtain maximum brewing value in the resultant extract, the hops must be extracted as soon as possible after harvest or before significant change in the α-acids content has occurred.

Table IV shows the resin analyses obtained on the liquid CO_2 extracts prepared in the pilot plant. Even with low α-acids hops such as the Hallertau Aroma variety, production of an extract with a high α-acids content is possible, and, in all cases, the LCV content of the extract is composed virtually of α-acids, LCV non-α-acids material being excluded in the extraction. Furthermore, the liquid CO_2 extracts produced from all varieties were completely soluble in n-hexane, indicating the absence of any hard resin material.

In addition to the experimental liquid CO_2 extracts, samples of two commercial extracts were examined, one made from the Oregon Bullion 1979 crop extracted with n-hexane and the other from the Hallertau Northern Brewer 1979 crop extracted with methylene chloride. The resin analyses for these samples are compared in Table V with those from the same hop varieties extracted with liquid CO_2.

In the two commercial extracts, the α-acids content and the ratio of α-acids to LCV are lower than in the liquid CO_2 extracts from the same hop variety. The divergence, as might be expected, is greater between the methylene chloride and liquid CO_2 extracts from the Hallertau Northern Brewer. However, the difference between the liquid CO_2 and solvent extracts is not as great as might be expected in view of the selectivity of the liquid CO_2, as demonstrated in

[3] Unpublished procedure.

TABLE III
Extraction of α-Acids and β-Acids with Liquid CO₂

Hop Variety	Extraction Efficiency, %		
	α-Acids[a]	β-Acids[a]	LCV[b]
Hallertau Aroma	99	102	78
Hallertau Northern Brewer	95	100	85
U.K. Northern Brewer	96	102	88
Wye Challenger	93	101	85
Oregon Bullion	94	95	80
Oregon Fuggle (enriched)	92	96	77
Washington Cascade (enriched)	94	103	78
Yakima Cluster	93	96	79
Pride of Ringwood, 1979	97	97	81
Pride of Ringwood, 1980	93	95	80

[a] By high performance liquid chromatography.
[b] Lead conductance value.

TABLE IV
Resin Composition of Liquid CO₂ Hop Extracts

Hop Variety	α-Acids (% w/w)	β-Acids (% w/w)	LCV[a]
Hallertau Aroma	37.3	35.1	36.4
Hallertau Northern Brewer	43.5	20.9	45.0
U.K. Northern Brewer	37.7	19.4	38.6
Wye Challenger	34.8	22.9	35.1
Oregon Bullion	38.3	22.7	38.3
Oregon Fuggle (enriched)	34.6	31.1	35.8
Washington Cascade (enriched)	32.8	38.7	32.7
Yakima Cluster	44.2	32.8	44.1
Pride of Ringwood, 1979			
Pilot	45.9	26.8	45.0
Commercial	44.9	25.6	46.8
Pride of Ringwood, 1980			
Pilot	41.9	22.9	40.9
Commercial	42.8	26.4	43.7

[a] Lead conductance value

TABLE V
Comparative Analysis of Liquid CO₂ and Solvent Extracts[a]

Extract Type and Solvent	α-Acids (% w/w)	β-Acids (% w/w)	LCV[b]	n-Hexane Solubles (% w/w)
Oregon Bullion				
Liquid CO₂	38.3	22.7	41.0	100
Organic Solvent (n-hexane)	36.2	20.5	40.7	99.5
Hallertau Northern Brewer				
Liquid CO₂	43.5	20.9	45.0	100
Organic Solvent (methylene chloride)	39.6	22.1	43.3	95.2

[a] Comparison of same varieties and same year but not of same lots.
[b] Lead conductance value.

Tables II and III. This could be explained by the fact that different lots, albeit from the same variety, were used for the two extracts. If hops of higher initial α-acids content, extracted soon after harvest, were used to prepare the commercial solvent extracts, one might expect to obtain an extract higher in α-acids content and lower in

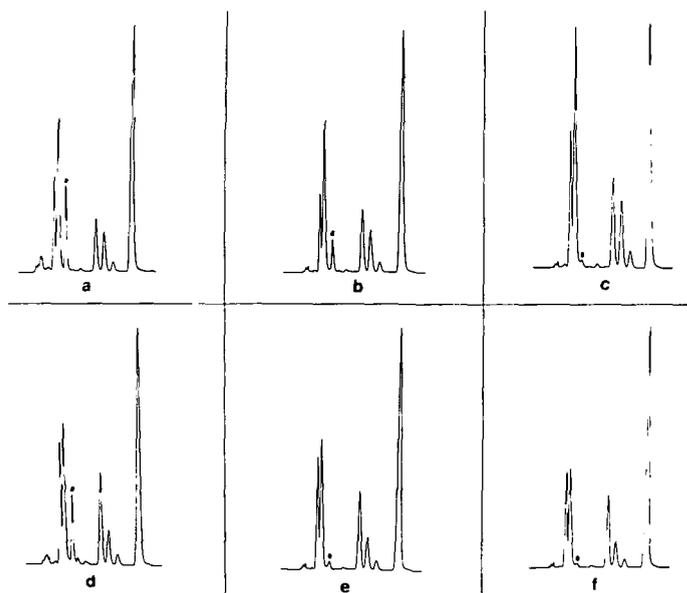**Fig. 1.** High performance liquid chromatograms of α-acids and β-acids in: a, Hallertau Northern Brewer hops; b, a commercial solvent (methylene chloride) extract of Hallertau Northern Brewer hops; c, a liquid CO_2 extract of Hallertau Northern Brewer hops; d, Oregon Bullion hops; e, a commerical solvent (n-hexane) extract of Oregon Bullion hops; f, a liquid CO_2 extract of Oregon Bullion hops. * = Hard resin component (possibly xanthohumol).
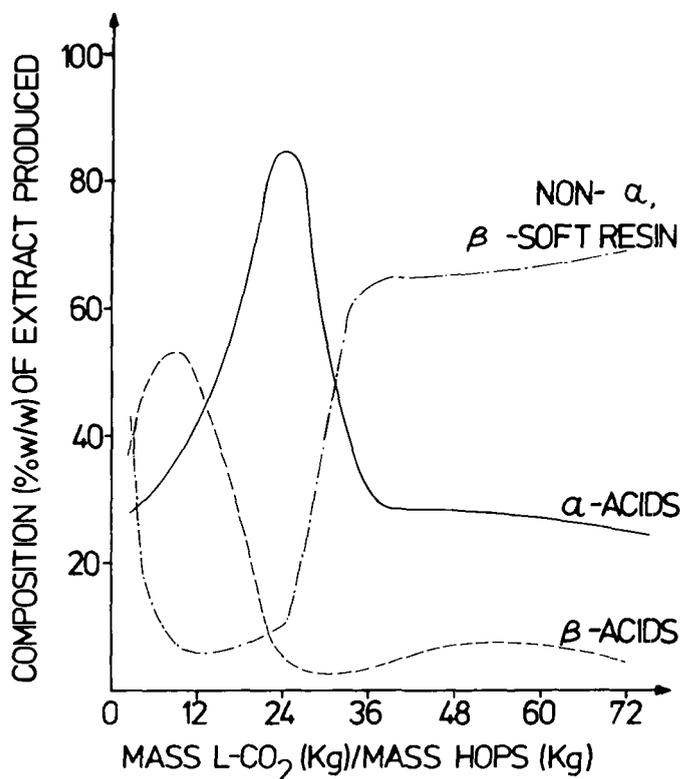

Fig. 2. Variation of liquid CO_2 extract composition with increasing extraction using a 5-m column and an extraction time of 6 hr.

LCV non-α-acids material.

The greater selectivity of liquid CO_2 as a solvent is further demonstrated by a comparison of the HPLC chromatograms obtained in the analyses of the α-acids and β-acids. Figure 1 indicates that, apart from the α-acids and β-acids and the internal standard, only one other major component is present. This component is present in highest relative concentration in the hops, still evident in significant quantity in the commercial methylene chloride extract, present in much reduced quantity in the commercial n-hexane extract, and virtually absent from the liquid CO_2 extracts. The identity of this component has not been confirmed, but it is a component of the hard resin fraction, possibly xanthohumol.

Essential Oil Composition of Extracts

The Brewing Research Foundation (2) has already demonstrated that the essential oil is preferentially extracted by the liquid CO_2, followed by the β-acids and then the α-acids. Obtaining a fraction rich in essential oil is thus possible.

The chromatographic effect referred to by Laws et al (6) was examined in the laboratories of Carlton and United Breweries Limited, using a long column formed by joining five of the pilot

TABLE VI
Essential Oil Analysis of Extracts

Hop Variety	Essential Oil Content, % v/w[a]	
	Commercial Extract	Pilot Liquid CO_2 Extract
Hallertau Aroma	...	5.4
Hallertau Northern Brewer	4.8 (Methylene chloride)	4.1
U.K. Northern Brewer	...	3.5
Wye Challenger	...	3.8
Oregon Bullion	4.9 (n-Hexane)	5.3
Oregon Fuggle (enriched)	...	4.6
Washington Cascade (enriched)	...	2.8
Yakima Cluster	...	2.8
Pride of Ringwood, 1979	...	3.9
Pride of Ringwood, 1980	4.1 (Liquid CO_2)	3.7

[a]Comparison of comparable but not identical lots.

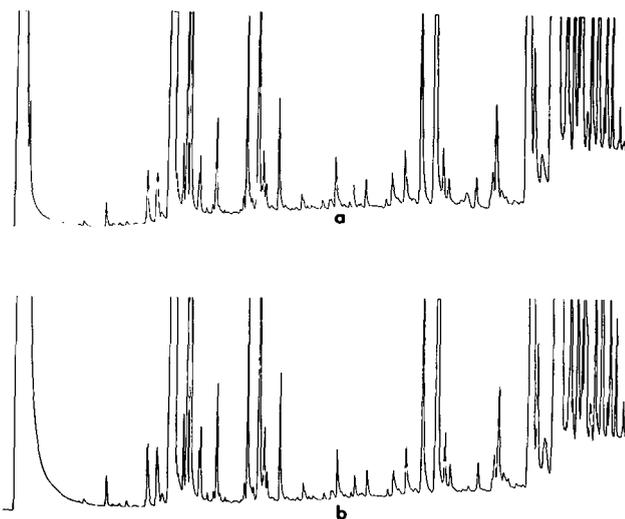**Fig. 3.** Gas chromatograms of vacuum steam distillates of: a, a liquid CO_2 extract of Hallertau Aroma hops; b, Hallertau Aroma hops.

plant columns in series. This experiment confirmed that the β-acids and α-acids, in that order, are recovered before any of the other soft resin material, with the exception of the essential oil fraction, which is eluted first (Fig. 2).

However, with the pilot plant used to prepare the extracts, no recycle facilities were available for the CO_2, which was merely vented to atmosphere, resulting in the loss of some of the essential oil. Essential oil recoveries ranged from approximately 70 to 95% for individual extractions. However, in the commercial plant, where recycle facilities were available and the sole variety processed was Pride of Ringwood hops, results from the 1980 crop indicated approximately 95% recovery of essential oil. Analyses of the extracted hops indicated virtually no residual essential oil (less than 0.01% v/w). The essential oil contents of hop extracts prepared in both commercial and pilot plants are detailed in Table VI.

In addition to determination of total essential oil contents, the hops and hop extracts were subjected to vacuum steam distillation. The system was designed to prevent loss of the more volatile constituents of the essential oil such as might occur with normal distillation procedures. This was assured by having a second trap in series before the pump which, on examination, was found in all cases to contain no essential oil constituents at completion of the distillation. The resultant essential oil isolate was examined by gas chromatography.

All isolates of liquid CO_2 extracts examined, including those in which the recovery of the essential oil on extraction with liquid CO_2 was less than 90%, gave gas chromatograms that did not differ

significantly from chromatograms of isolates from the corresponding hops. This similarity indicated that no significant fractional volatilization of the essential oil had occurred during CO_2 venting.

The gas chromatograms of the essential oil isolates from the Hallertau Aroma hops and the corresponding liquid CO_2 extract are shown in Fig. 3. Figures 4 and 5 show, for comparison, gas chromatograms of essential oil isolates from the Hallertau Northern Brewer solvent and liquid CO_2 extracts and the Oregon Bullion solvent and liquid CO_2 extracts, respectively.

Figure 6 demonstrates the similarity of the trace obtained from isolates of Pride of Ringwood extracts prepared in the pilot and commercial liquid CO_2 plants.

Liquid CO_2 Extract Stability

Previous investigations (5) have demonstrated the long term stability of extracts prepared with liquid CO_2. Obviously, no data

TABLE VII
Liquid CO_2 Extract Stability

Storage Time (days)	Content (% w/w) at °C					
	α-Acids			β-Acids		
	20	30	50	20	30	50
0	45.0	45.0	45.0	28.2	28.2	28.2
20	45.1	46.1	47.2	26.7	28.3	29.8
35	45.8	45.2	44.4	27.8	27.9	27.9
100	45.0	44.9	...	28.0	27.6	...
150	43.7	44.8	...	27.5	28.2	...
365	44.2	44.1	...	26.9	27.0	...

Fig. 5. Gas chromatograms of vacuum steam distillates of: a, a liquid CO_2 extract of Oregon Bullion hops; b, a commercial solvent (n-hexane) extract of Oregon Bullion hops.
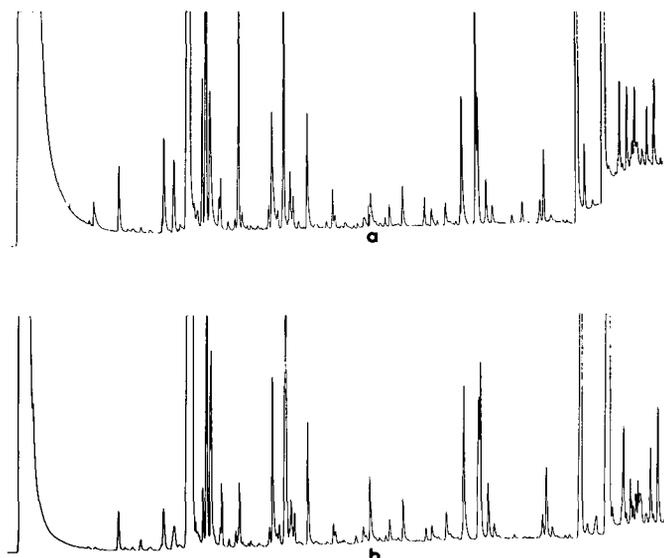

Fig. 4. Gas chromatograms of vacuum steam distillates of: a, a liquid CO_2 extract of Hallertau Northern Brewer hops; b, a commercial solvent (methylene chloride) extract of Hallertau Northern Brewer hops.
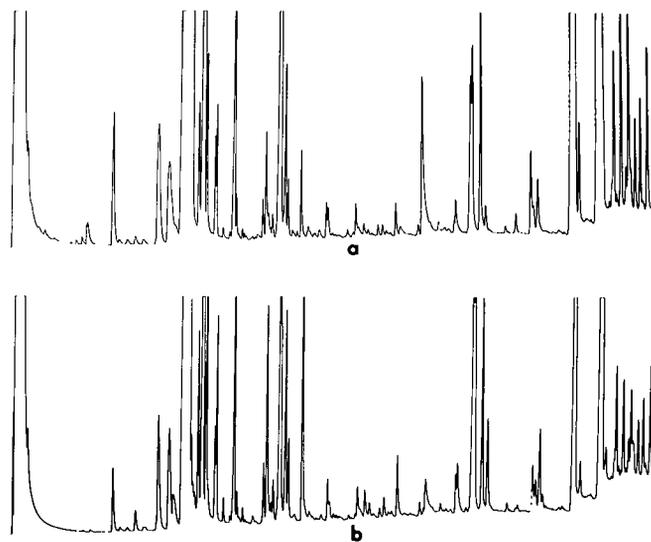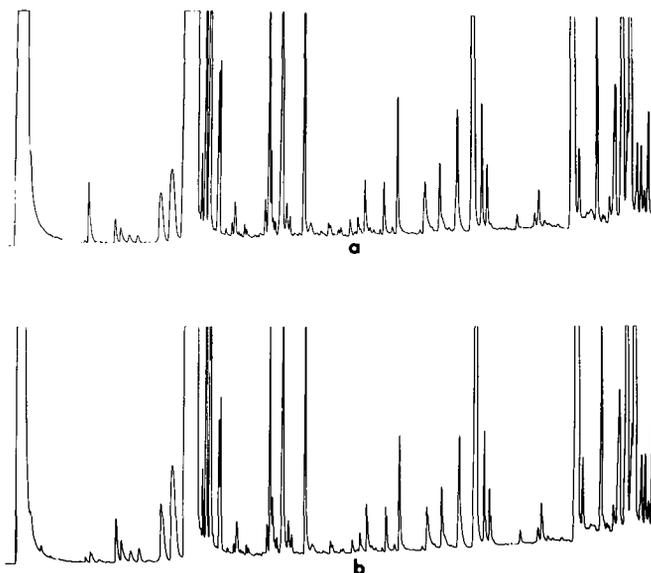

Fig. 6. Gas chromatograms of vacuum steam distillates of: a, a pilot plant liquid CO_2 extract of Pride of Ringwood hops (1980 crop); b, a commercial plant liquid CO_2 extract of Pride of Ringwood hops (1980 crop).

are now available on commercial liquid CO_2 extracts, but extracts prepared in the company's pilot plant have been under test at a range of temperatures for approximately twelve months. Both α-acid and β-acid contents have been monitored as sensitive indicators of chemical change during storage at 20, 30, and 50° C without any evidence of significant change over the period of the test (Table VII).

Isomerized Liquid CO₂ Hop Extract

An aspect of hop extract technology that confronts the manufacturer of liquid CO_2 hop extract is how best to produce an isomerized hop extract from such a material without using organic solvents. Carlton and United Breweries Limited is a committed user of isomerized hop extract and hence had to face this problem at the outset of the development of a commercial plant.

Currently two alternative proposals exist in the literature (4,6), both of which conduct the isomerization of the α-acids in the absence of solvent and before separation of the other components of the liquid CO_2 extract.

Because of concern about possible yield differences and flavor defects resulting from such an approach, the decision was made to isolate the α-acids from the total extract before isomerization. This has been achieved by the development of a new process, for which patent applications have been filed, whereby the extract is presented in a finely divided form to a solution of aqueous alkali. After isolation of the α-acids by this process, precipitation of the α-acids and isomerization are effected by means of a previously published process (I) whereby the magnesium complexes of the α-acids are formed and subjected to heat.

The other resin constituents and essential oils are recovered unchanged, for use as a base extract fraction, again under subambient temperature conditions in which minimal chemical change occurs and complete essential oil recovery is ensured. The fraction of the isomerized extract that contains isohumulone is obtained as a free-flowing, off-white powder containing 80–85% iso-α-acids on a dry weight basis.

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