Production of Solvent-Free Isomerized Extracts[1,2]

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ABSTRACT

Extraction of hops using liquid carbon dioxide provides a convenient method of obtaining a high-quality yellow product which usually contains over 90% of the available α-acids in the original hops. Under suitably controlled conditions, this solvent does not extract significant amounts of hard resins, fats, waxes, or pigmented materials. Brewing trials using these extracts gave beers of clean flavor which could not be distinguished by taste or analysis from control beers which were hopped in the usual way. Extracts obtained using liquid carbon dioxide can be converted into soluble isomerized extracts, in high yield, by a process involving three simple stages. The isomerized extracts produced in this way are free of residual organic solvents and can be added directly to bright beer without the formation of haze. The resulting beers had a satisfactory shelf life and showed no tendency to gush.

Key words: *Hop extracts, Isomerized extracts, Liquid carbon dioxide, Preparation.*

Substantial quantities of beer are now bittered by the replacement of kettle hops with either hop extracts or isomerized extracts. The various grades of commercially available hop extracts are now well established throughout the world as satisfactory substitutes for hops. Many of these products have the added advantage that, unlike hops, they can be stored for prolonged periods without loss of α-acids (2, 10). Isomerized extracts, which are used to best advantage to bitter beer after fermentation, are used to a more limited extent. The British brewing industry is a relatively large user of isomerized extracts but it is unusual for more than 40% of kettle hops to be replaced by this type of extract. However, certain American and Australian brewing companies use isomerized extracts to provide all of the bitterness in their beers.

The first stage in current methods for preparing both types of concentrates involves the extraction of hops with an organic solvent. Methylene chloride is now used extensively by commercial hop extractors, but hexane, trichloroethylene, and methanol also find a limited application. The nature and amount of hop components in the extracts vary with the organic solvent used. However, all of the solvents extract some fats, waxes, pigmented material such as chlorophyll, β-acids, and uncharacterized soft resins which have little or no brewing value, together with the desired α-acids. In addition, methanol and similar solvents also extract substantial quantities of polyphenols, hard resins, and certain water-soluble compounds of hops.

Hop extracts are obtained by evaporation to give products which normally contain low levels of residual solvent (8). During wort-boiling, residual solvents present in hop extracts are lost (4) with the steam and none can be detected in the final beer (31).

Preparation of isomerized extracts usually necessitates the use of a number of organic solvents such as toluene, methanol, methylene chloride, ethyl acetate, and isopropyl acetate, used either during the purification of fractions containing α-acids or in subsequent concentration of the iso-α-acids (1, 22). Isomerized extracts are normally added toward the end of the brewing process, permitting survival of residual solvents in the finished beer. It is important, therefore, to ensure that levels of residual solvents are low. Although at present there are no problems in producing both hop extracts and isomerized extracts which meet public health requirements with respect to levels of residual solvents, it is unlikely that there will be any relaxation of such requirements and stricter rules in future could give rise to difficulties.

Recently, a preliminary communication (18) described a method involving the use of liquid carbon dioxide for obtaining extracts which are rich in α-acids and free of residual organic solvents. This paper gives further details of this process, discusses the use of these extracts in brewing, and describes a simple method for producing isomerized extracts, in high yield, without using organic solvents.

EXPERIMENTAL

Extraction of Hops with Liquid Carbon Dioxide

Portions of powdered hops (100–200 g) were extracted with liquid carbon dioxide using the apparatus shown in Fig. 1 following a published procedure (18). A number of different varieties of hops were extracted using this technique; the results are shown in Table I.

Preparation of Isomerized Extract

Powdered Northern Brewer hops (200 g), which contained 6.3% of α-acids, were placed in the column of the extractor shown in Fig. 1 and extracted with liquid carbon dioxide as previously described (18). A further five portions of hops (200 g) were extracted in this way and the extracts were bulked (169 g). Examination of the

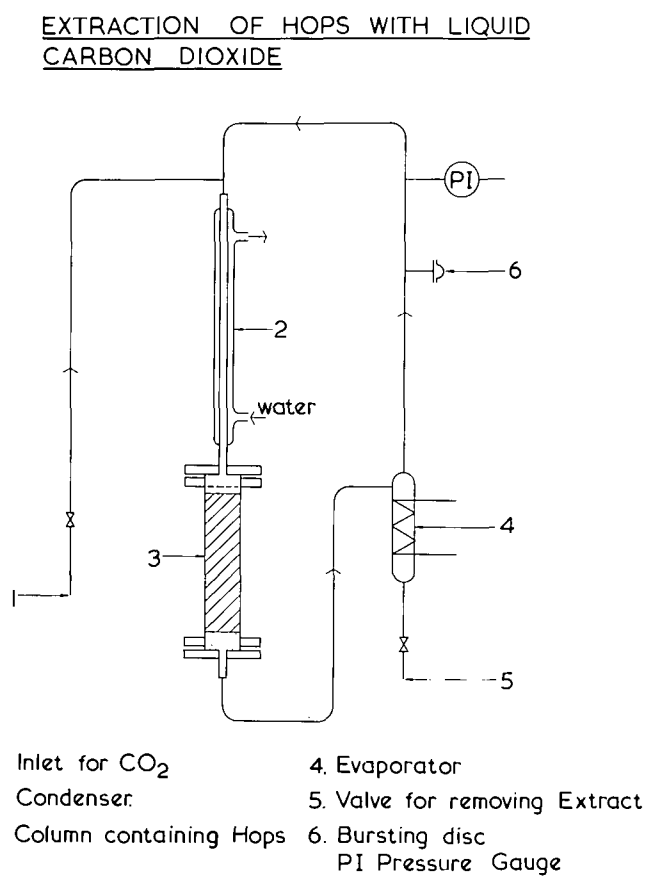

Fig. 1. Extraction of hops with liquid carbon dioxide (CO_2).

[1]Parts of this work are the subject of patent applications.
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extract by a conductometric procedure (17) showed that it contained 43% of α-acids and calculations showed that 96.1% (72.6 g) of the available α-acids (75.6 g) had been extracted from the hops.

A portion of the extract (28 g) was placed in a flask fitted to a condenser via a suitable trap. Sodium carbonate (5.0 l., 0.1 *N*) was added and the mixture was boiled for 20 min under an atmosphere of nitrogen. The hop oil which collected in the trap was discarded and the mixture rapidly cooled to *ca.* 0°C. The pH of the solution was adjusted to 4.0 by the addition of hydrochloric acid (2*N*), and β-acids immediately started to precipitate. The mixture was stirred at this pH for 1 hr, 100 g kieselguhr (diatomaceous earth) was added and a stream of nitrogen was passed through the mixture for 15 min to aid flocculation of the β-acids. The mixture was filtered through a bed of kieselguhr (150 g) which was washed with water (40 ml). The filtrate and washings were combined and the pH of the solution adjusted to 9.0 by the addition of aqueous potassium carbonate (2*N*) to give a dilute solution of the isomerized extract, which was concentrated to 1000 ml by rotary evaporation at reduced pressure (bath temperature 50°C, 15 mm Hg). A qualitative examination of the extract by thin-layer chromatography (6) showed the presence of *cis*- and *trans*-iso-α-acids but only trace quantities of α-acids and β-acids. A quantitative analysis of this extract using column chromatography on Sephadex (23) showed that it contained 1.1% iso-α-acids. Hence 91.4% of the α-acids present in the extract obtained using liquid carbon dioxide was converted into iso-α-acids. The extract was concentrated to 15% iso-α-acids (w/v) by rotary evaporation at reduced pressure (bath temperature 50°C, 15 mm Hg) without loss of iso-α-acids. Therefore, in this example, 87.8% of the initial α-acids present in the hops was in the isomerized extract in the form of iso-α-acids.

A similar procedure was used to obtain isomerized extracts from Wye Northdown, Wye Target, and Comet hops (see Table II).

Analysis of Hops and Extracts

Lead conductance values were used as a guide to levels of α-acids in hops (14) and extracts (17). β-Acids and iso-α-acids were estimated by column chromatography on Sephadex (23). Hops and extracts were assayed for essential oils using the procedure described by Howard (12).

Beer Production

Beers were produced on a pilot scale from an all-malt grist using a standard procedure (21). Analytical values for beers produced using hops and extracts are shown in Table III (7).

RESULTS AND DISCUSSION

Liquid carbon dioxide has found extensive application for extracting volatile substances from natural products (27, 28). The extraction of hops with this solvent has received the attention of Russian and Japanese investigators. During the last 12 years, studies using liquid carbon dioxide have led to the production of over 5,000 kg of hop extract in Russia (26). Pekhov and coworkers (25) described the production of an anhydrous light-brown-colored hop extract which dissolved completely in ethanol. Using liquid carbon dioxide it was possible to remove over 90% of the total extractables from hops after 2 hr (24), but the resulting extracts consisted of a complex mixture of hop components (29). Such extracts were not very stable and extensive oxidation occurred after storage for 9 months at ambient temperature together with an increase in the average size of particulate material. Recently, Japanese investigators described methods for extracting hops with liquid carbon dioxide which gave extracts which were rich in both resins and tannins (15). The use of carbon dioxide in the supercritical state to extract hops is reported (5, 30) to give olive-green-colored extracts of complex composition, and it is clear that, even under these conditions, extraction of hop resin constituents was not very selective.

In contrast selective extraction of certain hop constituents was found to occur if hops were extracted at ambient temperature (15° to 20°C) with liquid carbon dioxide. The resulting extracts consisted largely of α-acids, β-acids, essential oil, and moisture. Portions of powdered hops can be conveniently extracted using a semicontinuous extraction process (18), shown diagrammatically in Fig. 1. Results of extractions of a number of different varieties of hops are given in Table I. The liquid carbon dioxide extracted 81–100% of the available α-acids, 56–91% of the β-acids, and 66–88% of the available oils. Longer extraction times probably would have resulted in a greater degree of extraction of α-acids from the samples of Wye Target hops. All of the extracts shown in Table I had a pronounced smell of fresh hops. They were essentially free of hard resins, containing only small quantities (<0.2%) of material which was insoluble in light petroleum. The extracts were golden yellow in color and clearly did not contain chlorophyll. Examination using a modification of the thin-layer chromatographic method used to examine malt (20) failed to reveal the presence of polyphenolic compounds in any of the extracts. Crystals of both α-acids and β-acids separated when these extracts were cooled to *ca.* 4°C.

Results of limited storage studies using extracts made from Wye Northdown hops are shown in Table IV. The α-acids content of the extracts was estimated by a lead conductometric procedure (17) and no significant loss of α-acids occurred after 8 months of storage in the cold (0°–4°C) or after 6 months at ambient temperature (10°–32°C). A visual examination of the extracts after storage did not reveal any changes in color or in the size of particulate material. Clearly these extracts are much more stable than those described by the Russian investigators (29).

Brewing Studies

Results of a number of brewing trials using hops and extracts are shown in Table III. In these studies beers were brewed on the pilot scale using a standard brewing procedure (21) and the hops or

TABLE I
Extraction of Hops Using Liquid Carbon Dioxide

Variety	Extraction Time hr	Weight of Hops Extracted g	Weight of Extract g	Available Hop Components Extracted		
				α-Acids %	β-Acids %	Oil %
Northern Brewer	5	200	26.3	96	87	67
Bullion	3	174	27.6	100	91	...
Comet	3	151	19.8	95	60	71
Wye Northdown	2	100	15.4	92	81	83
Wye Target A[a]	1	143	19.8	81	56	86
Wye Saxon	3	134	18.9	90	83	66
Wye Target B[a]	1	121	18.7	81	...	88

[a]A and B are two different growths of Wye Target Hops.

extracts were added at the start of wort-boiling.

A comparison was made of the brewing performance of Wye Northdown hops using one portion added directly in the copper and a similar portion of hops as liquid carbon dioxide extract. The two beers were generally very similar (see Table III), both showing normal head retentions and having satisfactory shelf lives. There was no significant difference when the flavors of these beers were compared using a two-glass taste test involving 48 tasters (3). Further trial extracts were prepared from hops of two different growths of Wye Target. Table III shows results of comparisons of the brewing performance of each growth of Wye Target hops with the corresponding extract. Clearly the analytical values of these beers were very similar and two-glass taste tests did not show any difference in their flavors.

As expected, extraction of hops with liquid carbon dioxide gave a product which can be used with confidence as a direct replacement of kettle hops. Extracts made in this way have the added advantage that they are free of residual organic solvents.

TABLE II
Conversion of Hop Extracts into Isomerized Extracts

Variety	Hop Extract		Isomerized Extract		
	α-Acids content %	Weight isomerized g	Iso-α-acid content %	Weight kg	Yield of iso-α-acids %
Northern Brewer	43.0	28.0	1.1	1.0	91.4
Wye Northdown	43.5	1.1	0.38	0.1	79.4
Comet	39.9	12.2	0.34	1.0	69.8
Wye Target	54.0	10.0	0.40	1.3	96.3
Northern Brewer	46.0	2.5	0.30	0.37	96.5

TABLE III
Brewing Studies Using Hops and Extracts

Variety	Addition α-Acids mg/l.	Wort Bitterness EBU	Beer Bitterness EBU	Use %	Shelf Life Weeks	Head Retention sec
Northdown Hops	105	44	27.0	25.7	15	104
Extract	105	43	28.4	27.0	25	103
Target, Growth A Hops	119	47	29.5	24.8	>11	107
Extract	118	55	29.7	25.2	>11	115
Target, Growth B Hops	119	43	28.3	23.8	> 8	110
Extract	118	54	29.1	24.7	> 8	113

TABLE IV
Effect of Storage on Extracts Made from Wye Northdown Hops

Extract	Time of Storage Months	α-Acids Content on Receipt %	α-Acids Content after Storage %
A[a]	4	45.2	45.1
	8		44.7
B[b]	4	44.1	44.3
	6		44.0

[a]Sample A stored cold (0°–4°C).
[b]Sample B stored at ambient temperature (10°–32°C).

Isomerized Hop Extracts

In commercial processes for producing isomerized extracts that can be added directly to beer, the crude product obtained by extracting hops with an organic solvent has to be extensively purified to give a fraction which is suitable for isomerization. To be usable, such fractions must be rich in α-acids but essentially free of fats, waxes, hard resins, β-acids, polyphenols, and pigmented materials such as chlorophyll. Attempts to directly isomerize solutions of a hop extract made with an organic solvent, in order to give a commercially useful product which can be added to fermented beer, have failed because degradation of fatty and waxy material often can give rise to off-flavors and the presence of β-acids and hard resins interferes with the dissolution of iso-α-acids. However, Humphrey (13) has recently shown that methylene chloride extracts of hops can be directly isomerized using magnesium oxide to give a product which can be used to bitter beer by addition to wort toward the end of boiling. Extracts produced by this process would be unsuitable for direct addition to beer because of the formation of excessive haze due to the presence of β-acids and hard resins.

A number of different methods are used to isomerize α-acids to give the more intensely bitter iso-α-acids. Substantial quantities of isomerized extracts are produced by effecting the isomerization in aqueous alkaline solution (11). High-grade products are also formed by refluxing α-acids in alcoholic solution in the presence of divalent cations (16). More recently Hildebrand and coworkers (9) found that, when magnesium salts of α-acids are heated, isomerization occurs to give high yields of iso-α-acids.

Solvent-Free Isomerized Extracts

Despite the fact that extracts of hops obtained using liquid carbon dioxide can contain relatively high levels of β-acids, it has not been necessary to remove them prior to isomerization. Starting with a carbon dioxide extract of hops, a three-stage route has been developed for producing high-grade isomerized extracts. The first stage involves boiling the extract in aqueous sodium or potassium carbonate solution to isomerize the α-acids into iso-α-acids. During this process, most of the hop oil present in the extract is lost with the steam. If desired, the hop oil can be collected by condensing the vapors using an essential oil trap. The solution containing iso-α-acids, β-acids and a little hop oil is cooled and the pH is adjusted to 4.0 by the addition of hydrochloric acid. At this pH the β-acids are readily precipitated and their flocculation can be aided by the addition of a little kieselguhr. The mixture is filtered and the pH of the filtrate is adjusted to 9.0 by the addition of aqueous potassium hydroxide. The dilute solution of isomerized extract, which is pale yellow in color, can be readily concentrated at reduced pressure without loss of iso-α-acids; any remaining hop oil is lost during this procedure.

A number of different varieties of hops have been extracted with liquid carbon dioxide and converted into isomerized extracts by the new process. Examination of these extracts by column chromatography (23) showed (see Table II) that the isomerization of iso-α-acids was generally very efficient. This method of analysis failed to reveal the presence of any α-acids, β-acids, or humulinic acid, but a thin-layer chromatographic examination (6) did reveal the presence of trace amounts of these compounds. However, examination of commercial isomerized extracts by these techniques usually revealed much higher levels of these undesirable compounds. The stability of isomerized extracts, made by the new process, on prolonged storage, is at present being investigated.

Products obtained by extracting hops with liquid carbon dioxide would be suitable starting materials for isomerization by other methods reported in the literature (9,16). However, it would normally be necessary to use organic solvents during processing and, consequently, the resulting isomerized extracts would contain some residual solvent.

Additions to Beer

Haze generally forms when commercially available isomerized extracts are added to bright beer. Hence it is normal practice in Britain to add these extracts to beer in conditioning vessels or to feed them into a beer main prior to final filtration. The formation of haze is normally accompanied by a loss of bitter substances from the beer. However, Table V shows that isomerized extracts (25 mg/l. iso-α-acids added) made by the process could be added to bright, unhopped beer without the formation of haze. There was no increase in haze values on pasteurization and the resulting beers had satisfactory shelf lives and showed normal head retentions. However, a substantial amount of haze formed when two commercially available isomerized extracts were added to unhopped beer in a similar fashion (Table V).

Gushing is a problem which is still occasionally encountered in beers bittered with isomerized extracts. Unhopped bright beers were treated with a number of different isomerized extracts to give a final concentration of *ca.* 25 mg/l. of iso-α-acids. These beers were then subjected to an induced gushing test (19) which is used in many British breweries. All of the beers treated with isomerized extracts made by the new process passed this rigorous test. However, gushing occurred when the crowns were removed from bottles of beer similarly treated with the commercial isomerized extracts (Table V). The particulate material present in these beers probably initiated the release of carbon dioxide.

Studies using isomerized extracts made from Northern Brewer hops have shown that the use of iso-α-acids was 87.3%. Hence the overall use of the α-acids in the hops to iso-α-acids in the finished beer was very high at 80.8%. The beers treated with the commercial isomerized extracts were filtered to remove haze before estimating the analytical bitterness. The use of iso-α-acids was just over 75% for the two beers. In these studies on use, a European Bitterness Unit (EBU) was assumed to correspond to 1 mg/l. of iso-α-acids. However, when the new extract is added to bottles of bright beer, the use must approach 100%.

Extraction of hops with liquid carbon dioxide followed by direct isomerization provides an attractive route for preparing high-quality isomerized extracts which are free of residual organic solvents. Brewing studies have shown that extracts produced by this new process are superior to those which are at present commercially available. Preliminary data suggest that the cost of producing both hop extracts and isomerized extracts using these new processes is likely to be substantially less than in existing plants.

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TABLE V
Addition of Isomerized Extracts to Bright Beer

Hops Processed	Iso-α-Acids Added mg/l.	Haze		Induced Gushing[a]	Beer Bitterness	Use
		Initial EBC units	after Addition EBC units	Beer Loss g	EBU[b]	%
Northern Brewer	24.7	0.52	0.55	None	...	...
Comet	24.8	0.52	0.52	None	20.5	82.7
Wye Target	25.1	0.52	0.53	None	...	...
Northern Brewer	24.5	0.52	0.52	None	21.4	87.3
Commercial extract Sample I	25.3	0.52	3.8	69.7	19.1	75.5
Commercial extract Sample II	25.0	0.52	4.1	78.2	18.9	75.6
Control[c]	...	0.52	...	0.5	...	...

[a]Bottles contained 278 ml beer.
[b]EBU = European Bitterness Unit.
[c]Unhopped beer.

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