A Rapid Solvent Extraction Method for Hop Essential Oils

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A solid-phase rapid solvent extraction method for hop essential oil is described. The principle of the method is based on the selective retention of the bittering components on the adsorption alumina, which was premixed with aqueous potassium hydroxide solution, while the essential oil is eluted. An individual extraction can be completed in 2 h. For increased efficiency, multiple extractions can be performed. Results show that the recovery of flavor components from hops is significantly more efficient with this extraction method than with steam distillation, especially for the oxygen-containing compounds or compounds with lower volatility. Extracts obtained by this method are suitable for direct gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS) analysis. Other aspects of the method are also discussed.

INTRODUCTION

The most common methods for isolating essential oils from hops are based on steam distillation, either under normal atmospheric pressure (Wright and Connery, 1951; Howard and Slater, 1957; Maule, 1966; Likens and Nickerson, 1967; Howard, 1970; Kunitake and Yada, 1973; Tressl et al., 1978; Sharpe and Laws, 1981) or under vacuum (Pickett et al., 1975, 1977; Laws et al., 1978). Less popular methods are extraction with organic solvents of various polarities (DeMets and Verzele, 1968; Laws, 1981), with liquid carbon dioxide (Laws et al., 1977), or with carbon dioxide under super critical conditions (Vitzthum et al., 1976; Muller, 1980).

Major disadvantages of these methods are length of operation, usually from 3–6 h or more; the need for special equipment, especially in the case of vacuum steam distillation or liquid carbon dioxide extraction; thermal degradation (Sharpe and Laws, 1981); and the incomplete recovery of compounds that are more water soluble and/or less volatile (Maule, 1966). Moreover, significant amounts of both α- and β-acids appear in the hop oil (Maule, 1966; DeMets and Verzele, 1968). The vacuum steam distillation method had a lower degree of thermal degradation for various compounds and did not carry over any of the α- and β-acids, but the water emulsion thus collected was unstable in storage and could not be analyzed directly without further sample preparation.

The goal of this study was to develop a rapid and simple solvent extraction procedure for the isolation of hop essential oils. This proposed method was expected to provide the following advantages: The essential oil should be free from any bittering components. The sample size for hops should be small, preferably around 10 g. The extracts should be ready for direct analysis with either gas chromatography (GC), gas chromatography–mass spectrometry (GC–MS), or both. The method should be time, cost, and space efficient; i.e., each extraction should be completed in 2 h, and multiple extractions should also be possible. Only general laboratory glassware and equipment that is easy to assemble and operate should be used. The method should also have the capability of being standardized.

EXPERIMENTAL SECTION

Rapid Solvent Extraction Method. Nugget, Cascade, Galena, Styrian, and Willamette hops were harvested from the experimental hop yard of Oregon State University in the fall of 1984. Hops were dried and stored frozen until analyzed.

An all-glass system (Figure 1) was used in this study. The chromatographic column (Kontes Scientific Glassware/Instruments, Vineland, NJ) was 330 mm in length and 22 mm in inner diameter. In the following order, 5 g of anhydrous sodium sulfate, 30 g of adsorption alumina, 80–200 mesh (Fisher Scientific Co., Fair Lawn, NJ), that had been premixed with 2 mL of 40% aqueous potassium hydroxide solution, 10.0 g of finely pulverized hops, and another 5 g of anhydrous sodium sulfate were added to the column. The modified alumina was prepared by stirring the mixture of alumina and aqueous potassium hydroxide solution thoroughly with a glass rod, followed by conditioning for 5 min. An aliquot of a stock solution containing 1.0 mg of naphthalene (internal standard) in pentane was added and mixed with the hops. The hops were then added in several portions and packed tightly into the column. Pentane, 300 mL, was used to elute the column at a flow rate of 20 mL/min with gentle suction. Solvent was removed in a rotary evaporator under slight vacuum and with a bath temperature maintained at 25 °C. Wax compounds were precipitated and removed by the addition of 1 mL of acetone, followed by filtration through a membrane filter.

A recovery study was conducted with the same instrumental setup, except that 100.0 mg of the hop oil from steam distillation along with 1.0 mg of naphthalene was applied to the column, instead of pulverized hops.

For each hop sample, three replicate extractions were analyzed and the results were averaged. All extracts were analyzed by capillary gas crhomatography (Cap-GC).

Hop Oil from Steam Distillation. Hop oil was isolated by using the method of Likens and Nickerson (1967). A 1.0-mg portion of naphthalene was added to 100.0 mg of hop oil, to which hexane was added to make a 1.0-mL solution. Samples were analyzed by Cap-GC.

Gas Chromatography. A Hewlett-Packard Model 5830 GC with a Model 18835 B capillary inlet system was used with a flame ionization detector. A Hewlett-Packard Model 18850A GC terminal was used for data reduction and peak identification. A 1 mm × 30 m glass open tubular column wall coated with SP1000 (Supelco Inc., Bellafonte, PA) was used to chromatograph all of the hop oil and hop extract samples. Helium was the carrier gas, with a head pressure of 1.1 kg/cm^2. The split ratio was 1:100. Oven temperature was programmed from 60 to 175 °C at 5 °C/min and then at 0.5 °C/min to 190 °C, with a 5-min hold at the initial temperature. The injection port temperature was 230 °C, and the detector temperature was 250 °C.

Peak identification was based on the comparison of relative retention times between the authentic compounds and the internal standard, and with standard addition procedure.

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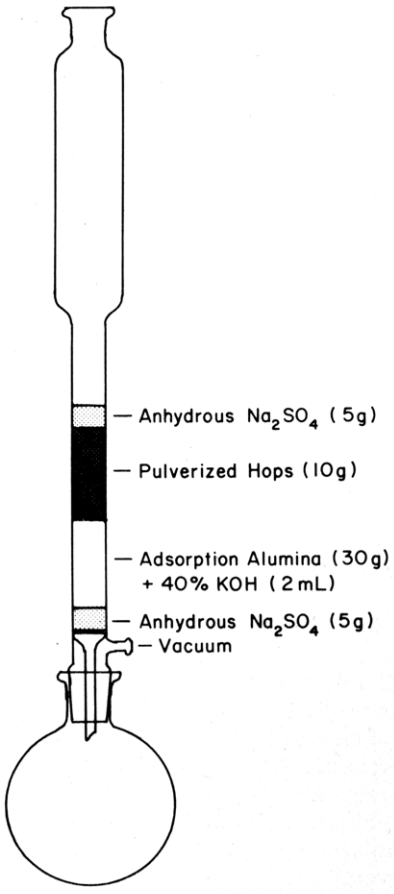

Figure 1. Equipment setup of the rapid solvent extraction (RSE) method.

Ultraviolet Spectrometry. A Hitachi Model 110 UV–vis spectrophotometer and the official analytical method of the American Society of Brewing Chemists (1976) were used to monitor the presence of both α- and β-acids in all hop extracts obtained by this solvent extraction method.

RESULTS AND DISCUSSION

Hop varieties harvested in 1984 were selected for studying the rapid solvent extraction (RSE) method. Whole hop cones were used to isolate the essential oil by steam distillation. Other samples were finely pulverized, and the essential oil was obtained by the RSE method. Evaluation of the efficiency and applicability of the RSE method was based on the comparison of chemical composition of hop oil obtained by the new extraction method and that from steam distillation.

Typically, the pulverized hops are extracted with pentane (Figure 1). The bittering components, α- and β-acids, are selectively retained on the potassium hydroxide treated adsorption alumina, while the essential oils are eluted.

Nugget hop oil from steam distillation served as the reference in a recovery study. The same hop oil was spiked to the column and recollected by the RSE method. Quantitation of selected compounds show that they are recovered satisfactorily by the new method. Among the three replicate samples, the coefficient of variation ranges from 2 to 6% for compounds monitored.

The performance of the RSE method was evaluated by processing selected pulverized hops. The composition of the hop oil (Table I) from this extraction method was then compared with the oil from steam distillation.

The amounts of flavor components in the extract by the RSE method are significantly higher than those from steam-distilled oil. Even myrcene, one of the most volatile

Table I. Rapid Solvent Extraction (RSE) of Selected Hops

	Nugget		Cascade		Galena		Styrian		Willamette	
	SD[a]	RSE[b]	SD	RSE	SD	RSE	SD	RSE	SD	RSE
myrcene	513.80[c]	528.30 ± 12.60	388.22	391.11 ± 12.31	228.29	273.88 ± 21.01	91.63	159.84 ± 2.33	240.59	242.73 ± 5.07
linalool	14.66	16.97 ± 0.05	4.07	3.79 ± 0.09	2.18	2.97 ± 0.38	1.37	3.97 ± 0.07	4.69	6.22 ± 0.05
β-caryophyllene	70.38	197.06 ± 0.94	32.13	46.19 ± 0.66	39.09	90.16 ± 4.36	32.88	75.88 ± 0.86	41.09	66.05 ± 1.40
farnesene	1.32	2.03 ± 0.09	45.50	80.04 ± 0.61	0.57	0.98 ± 0.11	24.04	63.19 ± 0.81	35.23	69.06 ± 5.32
α-humulene	179.12	413.97 ± 13.71	87.04	114.20 ± 1.76	84.29	177.49 ± 8.46	106.73	230.84 ± 2.23	123.15	178.69 ± 6.44
α-terpineol	5.12	13.26 ± 0.65	3.07	4.06 ± 0.02	4.16	7.68 ± 0.33	4.08	7.99 ± 0.97	1.67	5.45 ± 0.95
citral	9.84	22.63 ± 0.99	6.52	9.10 ± 0.66	5.60	12.67 ± 0.46	2.72	5.52 ± 0.07	3.29	4.84 ± 0.36
geranyl isobutyrate	6.93	18.35 ± 0.58	12.03	17.87 ± 0.15	9.74	19.82 ± 0.58	2.33	5.33 ± 0.15	2.33	3.92 ± 0.44
geraniol	0.58	1.02 ± 0.20	0.92	0.91 ± 0.04	0.44	1.00 ± 0.04	0.78	0.65 ± 0.15	0.25	0.81 ± 0.13
caryophyllene oxide	0.65	0.88 ± 0.05	0.51	2.90 ± 0.31	0.45	5.41 ± 0.63	1.14	1.78 ± 0.15	0.14	0.91 ± 0.12
humulene monoepoxide I	0.28	0.34 ± 0.05	0.26	1.17 ± 0.16	0.13	1.51 ± 0.09	0.43	1.00 ± 0.13	0.09	0.41 ± 0.06
II	2.16	3.29 ± 0.25	2.46	11.55 ± 0.89	1.17	15.61 ± 2.01	4.99	8.22 ± 0.89	0.56	3.75 ± 0.45
III	0.16	1.31 ± 0.04	0.41	0.36 ± 0.21	0.33	0.55 ± 0.09	0.88	1.31 ± 0.14	0.51	0.73 ± 0.04
humulenol II	0.04	0.80 ± 0.16	5.08	+[h]	0.31	1.43 ± 0.51	0.70	0.71 ± 0.02	0.24	0.37 ± 0.07
humulene diepoxide A[d]	0.43	4.52 ± 006	–[g]	+	0.1j	0.44 ± 0.06	1.37	5.58 ± 0.39	0.84	10.86 ± 0.74
B[d]	0.15	1.73 ± 0.41	–	+	–	0.84 ± 0.25	0.26	0.71 ± 0.27	–	0.64 ± 0.12
$\sum C_{15}H_{24}$[e]	249.50	611.03	119.17	160.39	123.38	267.65	139.61	306.72	101.37	244.74
$\sum C_{15}H_{24}O$[f]	3.87	12.87	8.21	15.98	2.53	25.79	9.77	19.31	2.38	17.67

[a] Hop oil from steam distillation. [b] Data reported as the average of three replicate extractions. [c] In mg/100 g of hops. [d] Tentatively identified. [e] Total amount of β-caryophyllene and α-humulene. [f] Total amount of oxidation products of β-caryophyllene and α-humulene. [g] Not detected. [h] Trace amount.

hop oil components, was recovered in slightly higher concentration by the RSE method as comparped to steam distillation. The quantitative increase in the recovery of the sesquiterpenes β-caryophyllene and α-humulene and their oxidation products by the RSE method is of particular importance. The sum of the amounts of β-caryophyllene and α-humulene in the hop oil from steam distillation as compared to the RSE extraction is 249.50 and 611.03 mg/100 g of hops for Nugget hops; 119.17 and 160.39 mg for Cascade hops; 123.38 and 267.65 mg for Galena hops; 139.61 and 306.72 mg for Styrian hops; and 101.37 mg and 244.74 mg for Willamette hops, respectively. When the RSE method is used, the combined recovery of the two sesquiterpenes is improved by a factor ranges from 1.35 to 2.41 with respect to the aforementioned hop varieties. The RSE method also enhanced the recovery of the oxidation products of β-caryophyllene and α-humulene by a factor ranging from 1.98 to 10.19 for Nugget, Cascade, Galena, Styrian, and Willamette hops, respectively. Besides, the UV spectrophotometric analysis of extracts obtained by the RSE method showed that all the α- and β-acids from hops are retained on the alumina.

The oxidation products of β-caryophyllene and α-humulene are believed to contribute the so-called "noble hop" or "kettle hop" aroma/flavor to beer (Tressl et al., 1978; Peacock et al., 1980; Fukuoka and Kowaka, 1983). It is important, therefore, to know accurately the amounts of these oxidation products, as well as their parent sesquiterpenes, in order to evaluate their hop contribution to beer flavor. Results have demonstrated that this rapid solvent extraction method is far superior to steam distillation in achieving this purpose.

The increased quantitative recovery of these oxidation products reflects the improved efficiency of the new method. In steam distillation, only compounds with sufficient volatility are vaporized from the hop mixture and collected. Both hydrophilicity and thermal degradation can significantly influence the chemical composition of the recovered hop oil. In the RSE method, the isolation of hop components depends mainly on the partition coefficients of various compounds between the eluting solvent and the hop matrix. The moisture level in the hop sample, around 8%, is so low that water solubility of compounds is of little consequence. Also, the extraction at room temperature eliminates thermal degradation.

A study shows that over 95% of the extractable essential oil components is recovered in the first 300 mL of pentane wash. Residual amounts of these components can be collected by further elution with more polar solvents, e.g., methylene chloride, ether, or methanol, with the drawback that a significant amount of bittering components is also eluted from the column simultaneously.

In comparison with the whole hops, the use of finely pulverized hops provides better results, owing to the increased surface area, which in turn improves the extraction efficiency. Care should be taken to prevent loss of essential oil prior to extraction, and therefore, hops are pulverized just before analysis or frozen in a closed container for short-term storage, if needed.

In order to retain the bittering components selectively on the adsorption alumina, it is necessary to premix the alumina with aqueous potassium hydroxide and to elute the essential oils with pentane. In preliminary investigations, other extraction systems had also been tested, including the use of solid potassium hydroxide and sodium carbonate, or aqueous sodium carbonate as the base; Celite or silica gel as the adsorbent; and polar solvents such as methanol, ether, and methylene chloride for elution. Results from these extractions showed that at best only partial retention of the bittering components could be expected. The data also suggest that hydrated ions from potassium hydroxide plus the surface properties of alumina provide sites of strong polar interactions. Most of the essential oil components are less polar (or more lipophilic), and their partition coefficients between the eluting solvent and the adsorbent are high enough so that the majority of these components will be eluted, independently of the polarities of the eluting solvent.

The situation for α- and β-acids is quite different. If both the polar and lipophilic interactions between the eluant and the α- and β-acids are competitive enough with the strong polar interactions between the α- and β-acids and the adsorbent, substantial amounts or even all of the bittering components will be eluted from the column along with the essential oils. This leads to an incomplete separation between the essential oils and the bittering components.

Certain groups of compounds, like the free fatty acids and enolizable compounds, will be partially or even totally retained on the column, due to the complexity in the composition of the hop essential oils and the polar nature of the treated alumina. When one tries to evaluate the efficiency of the RSE method on selected component(s) from the raw hops, the choice of the proper model system is crucial. Matrix effects play an important role in the results obtained in these investigations. When a standard pentane solution containing 1-octanol, methyl octanoate, methyl decanoate and geranyl isobutyrate is used, their recovery rates are 17, 81, 105, and 66%, respectively. Similarly, when hop oil from steam distillation is used, their recovery rates are 72, 89, 97, and 91%, respectively. Furthermore, the presence of bittering components enhances the recovery of various components from the whole hops when the RSE method is used. Data also suggest that competitive binding for the sites for strong polar interactions on the alumina occurs between the bittering components and the essential oil components. As the bittering components are strongly bound to the modified alumina, the interactions between the alumina and the essential oil components can be expected to be weakened considerably, thus making them more easily removed from the column. In addition, the ratio between the volume of pentane and the volume of either hops or alumina is 10 to 1; components with smaller partition coefficients in pentane can still be effectively extracted.

As many as 18 extractions can be processed within an 8-h period by a single operator, with only six sets of columns. More extractions can be handled if additional equipment is used. An individual extraction can be completed in 2 h. All extracts are immediately ready for direct analysis by capillary gas chromatography.

The rapid solvent extraction method is a simple, reproducible, and efficient method for the isolation of essential oils from hops. Results show that there is complete separation between essential oils and bittering components. The method is especially effective for recovery of compounds that are less volatile and/or compounds that are more wataer soluble. The simplicity of this method makes it labor and time efficient by making multiple extractions simultaneously. Since only general laboratory equipment and commercially available reagents are used, good agreement of the results can be expected from different laboratories. This method may be of great significance to studies involving the analysis of large numbers of hop samples. Accurate and reproducible results have been obtained consistently by this method using only a 10-g

sample, in contrast to the 200 g of hops required for steam distillation.

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Determination of Ethanol in Complex Products of Distilleries by Stripping and Gas Chromatographic Analysis

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This paper describes a method of volatile ethanol determination in complex products of distilleries, suitable for automated on-line analysis. Basically an inert gas bubbles through the alcoholic liquid phase and strips a small quantity of ethanol. The vapor phase is subsequently analyzed by gas chromatography with a flame ionization detector. We show that the analysis of the vapor phase permits a fast and reproducible determination of ethanol concentrations in the liquid phase ranging from 0.01 to 10% v/v. Periodic monitoring with standard test solutions is necessary.

INTRODUCTION

Ethanol has recently gained attention as an attractive energetic product, and it has become urgent to develop and to optimize its production.

Alcoholic fermentation of sugared juices produced from beets, sugar cane, grapes, molasses, grains, or corn leads to a wine containing 5–9% v/v of ethanol. The wine, after distillation, leaves ethanol as a main product (96% v/v) and byproducts such as vinasse (residue) that still contains 0.1% v/v of alcohol.

Optimal distillery production is obtained by monitoring processes and by establishing balances for controlling material and energy. It is important to know the ethanol content of the intermediary and final products at any time, in order to control and conduct more precisely the different production steps such as fermentation and distillation.

This paper deals with the study of ethanol analysis using a simple technique that easily leads itself to automation and on-line implementation.

Usually, automatic direct analysis of ethanol in the different liquid products using a specific electrode or gas chromatography is difficult due to particles in suspension. For other analyses in the plant laboratory such as ebulliometry or oxidation the results will only be known some 10–30 min after sampling. Reference methods that require the extraction of ethanol from a sample, usually by distillation, take about 2 h. Such methods are therefore not well suited for continuous monitoring of a production plant.

The following procedures for analyzing volatile substances in a variety of media have been reported in the literature:

The "tubing method" is applied to the measurement of dissolved ethanol in a yeast culture (Dairaku and Yamane, 1979), and also to oxygen, carbon dioxide, and methanol dissolved in culture liquids. For automatic and repeated analysis, the disadvantage of this method lies in the plugging of the tube by the particles that are present in the products.

The gas chromatographic headspace analysis (Hachenberg and Schmidt, 1979; Weurman, 1969) has been reported in different ways: (1) Direct sampling and chromatographic analysis of the atmosphere in equilibrium with the solid or liquid containing volatile substances permit qualitative and quantitative analysis of flavor components of fruits (Paillard et al., 1970) and water organic substances (Friant and Suffet, 1979). (2) After gas extraction from the sample, a concentrate of trace volatile substances is obtained by trapping them on activated carbon, porous polymers, cold traps, ... They are eluted, by thermal methods or with a solvent, into the gas chromatograph (Nuñez et al., 1984). This procedure covers many applications: dairy products (Morgan and Day,

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