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

Elisabeth D. Dumoulin,* Antonio C. Duarte-Coelho, Pierre O. Cogat,[1] and Jacqueline T. Guérain[1]

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,

Département Génie Industriel Alimentaire, Ecole Nationale Supérieure des Industries Agricoles et Alimentaires, 91305 Massy, France.
[1]Union Nationale des Groupements de Distillateurs d'Alcool, 75003 Paris, France.

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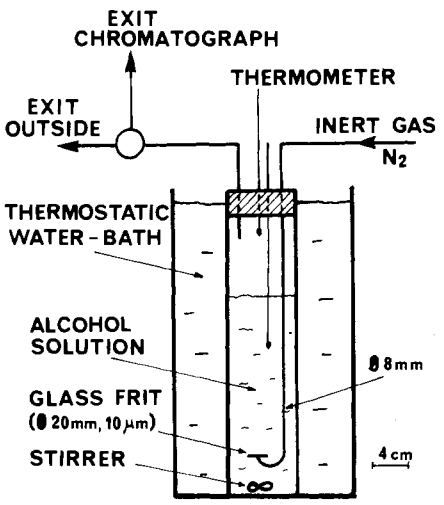

Figure 1. Apparatus.

1965), fruits (Ismail et al., 1981), wine or alcoholized beverages (Williams and Strauss, 1977; Noble et al., 1979). For the latter, water and ethanol are eliminated to analyze the other volatile components.

The principle of this technique may be retained although for high concentrations of volatile substances a trap is probably not necessary.

Different parameters influence the efficiency of stripping: temperature, gas flow rate, medium composition, salt addition (Furter, 1977).

These studies lead us to propose to strip volatile ethanol by bubbling an inert gas through the distillery product. The temperature and gas flow rate being maintained constant, the stripped ethanol quantity, evaluated by gas chromatography, is representative of the ethanol content of the product. We are able to analyze ethanol in a large variety of distillery products, ranging from 0.01 to 10% v/v. The equipment will be connected to the production line to obtain the ethanol composition of the liquid products at each step of fabrication.

MATERIALS AND METHODS

Stripping Apparatus. The main part of the apparatus consists of a cylindrical glass container that is only three-fourths filled with the sample to avoid priming (Figure 1). The temperature is kept constant by a thermostated waterbath (±0.3 °C). The nitrogen flow enters from the bottom of the container, through a glass frit, and bubbles through the sample. To ensure an efficient exchange between gas and liquid, bubble diameter and contact time are two important parameters (Richon et al., 1980). The trajectory of the bubbles in the liquid is longer than 22 cm, and the diameter of the bubbles is about 3 mm. The stirrer, by leading the bubbles in a spiral path, increases the contact time between gas phase and liquid phase.

The carrier gas strips the components into the vapor phase; the outlet gas flow is periodically injected into a chromatograph by means of a gas sampling valve. A water column placed on the outlet gas circuit maintains a constant pressure.

Gas Chromatography Instruments. An Intersmat IGC 120 chromatograph equipped with a flame ionization detector is fitted with a stainless-steel column packed with Porapak Q (1.8 m × 0.32 cm; 80/100 mesh). The oven operates at 185 °C; the injector and the detector operate at 215 °C. The N_2, H_2, and air flow rates were 20, 20, and 500 mL/min, respectively. The volume of the sampling loop is 1 mL.

The ethanol peak height or the area is determined (Integrator Intersmat ICR 1B).

The chromatographic conditions are maintained constant during all tests; only the sensitivity may vary.

The retention time of ethanol is thus less than 5 min. The detection limit is 0.001%.

Reagents. For calibration we use standard solutions prepared with absolute ethanol and distilled water.

To reproduce the salt matrix of the distillery products, we add K_2SO_4 (analytical reagent grade, Prolabo).

Thiomersal is used as a stabilizer (100 mg/L) and Bevaloid A as an antifoaming agent (0.2 mL/L).

A variety of different distillery products have been tested: grape and beet wines (7–10% v/v ethanol), beet vinasse (0.01%), beet wort during fermentation (0–9%), marc of grapes (2–3%).

Comparison with Other Analytical Methods. To check the validity of the technique, the experimental results about ethanol contents were compared with those obtained by standard methods: pycnometry and areometry for water/ethanol solutions or distillates obtained from complex products; ebulliometry, chemical oxidation of ethanol for low contents (2%).

RESULTS AND DISCUSSION

We have tested the experimental setup by means of standard water/ethanol solutions and studied the effect of temperature and gas flow rate on the detection sensitivity and reproducibility.

Subsequent tests on industrial samples were performed to define specific operating conditions, including the composition of standard solutions. We have examined the reproducibility of the results and compared them with those obtained with usual classical methods.

Finally, we have established the relationship between the ethanol concentration of the compounds detected by the chromatographic system and the concentration in the original matrix.

Effect of Stripping on the Losses of Ethanol for the Medium. We want to study the decrease of the ethanol content in the medium during stripping. The volatile solute, ethanol, passes from liquid phase to vapor phase through the interface of the bubbles in two steps: mass transfer in the liquid phase and then diffusion in the gas phase. If the bubbles are small and their trajectory in the liquid is long enough, we can assume that equilibrium is reached between the two phases.

In fixed operating conditions for carrier gas flow rate and temperature, the chromatographic peak height is related to the medium composition, with the following assumptions: the outlet gas is assimilated to water-saturated air; the ethanol content of the outlet gas phase is that of a phase in equilibrium with the studied liquid phase at that temperature.

From the ethanol and water molar fractions in the liquid phase, and the ethanol/water activity coefficients calculated with Van Laar constants (A_{12} = 0.67; A_{21} = 0.42 (Perry, 1963)), we deduce the ethanol and water molar fraction in the vapor phase, Y_1 and Y_2, respectively. In the vapor phase we have

$$\text{mol of water/mol of ethanol} = Y_2/Y_1$$

In 1 h, the stripped ethanol is

$$Z\ (\text{mol/h}) = \frac{1000}{22.4}\frac{273}{\Theta}DG\frac{M_g}{M_w}\frac{Y_1}{Y_2} \qquad (1)$$

or

$$Z'\ (\text{kg/h}) = ZM_e$$

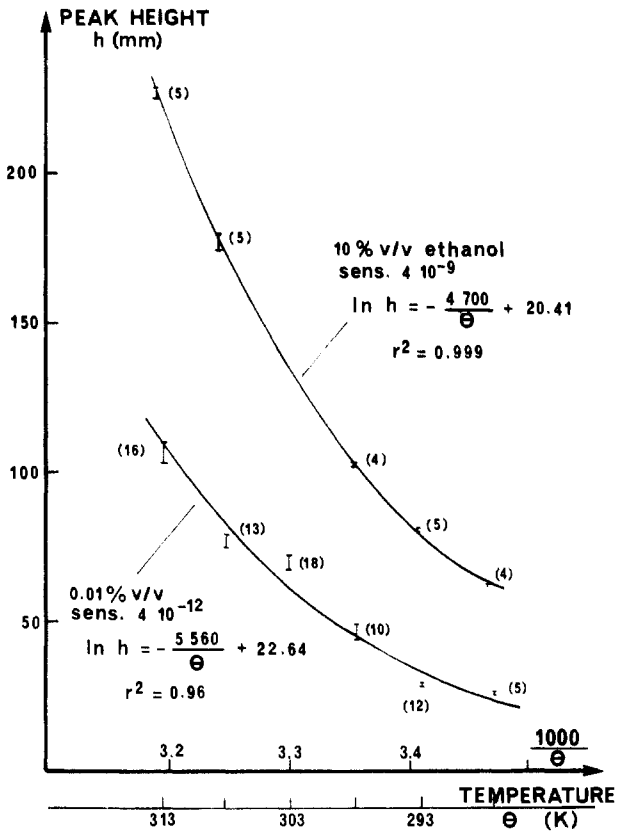

Figure 2. Influence of temperature on ethanol stripping (number of peaks in parentheses).

where Θ is the temperature (K), D is the nitrogen flow rate (m^3/h), and M_g, M_w, and M_e, are the molar weights of inert gas, water, and ethanol, respectively. G is the water content of the saturated air, at Θ (kg water/kg dry air).

The rate of decrease in ethanol content per hour, for the analyzed medium V (m^3), containing x% v/v ethanol, with a specific weight ρ (kg/m^3) may be expressed as

$$Z'/xV\rho = \%\ \text{ethanol lost/h}$$

To analyze a solution containing 0.01% v/v ethanol, if we sample 1 mL of the carrier gas at 20 °C, we analyze 3.7×10^{-10} mol of ethanol, corresponding to a chromatographic peak height of 29 mm in the above described operating conditions. With D = 600 mL/min and V = 0.9 dm^3, we calculate a loss of 1.1% for 1 h. During a test, with injections every 7 min, we observed no systematic decrease in the peak height: for 10 peaks, the average variation is about 2%.

We therefore conclude that it is possible to strip ethanol from a sample with the previously described operating conditions during a period sufficiently long for several injections, with reproducible results.

Effect of Temperature. In the range 15–40 °C, we observe constant peak heights during 1 h for one selected temperature, but the height varies strongly with a temperature variation.

From the variation of the activity coefficients with temperature we can calculate the variation with temperature of the quantity of stripped ethanol when the carrier gas flow rate is maintained constant. Calculations and experimental results suggest an exponential variation (Figure 2). This makes it possible to analyze small quantities: we try to obtain an increase in the ethanol content in the vapor phase for the best chromatographic conditions. The stripped ethanol quantity that increases with temperature leads to greater losses for the medium: 0.88% at 20 °C, 1.43% at 30 °C, 2.52% at 40 °C (per hour).

Furthermore, condensation of volatiles in the tubing and the valve must be prevented by insulating or heating this section. For the tests described in this paper, we chose a bath temperature 2 °C below the ambient temperature.

The temperature will be important during calibration. In the plant, it will be necessary to take into account the variation of the temperature according to the product.

Effect of Carrier Gas Flow Rate. As a carrier gas we retain nitrogen instead of air since the latter may favor proliferation of microorganisms.

From the relation (1), we deduce that in the volume v of the chromatographic loop we have $(Zv)/D$ moles of ethanol. According to the assumptions, Z is proportional to the carrier gas flow rate D; the analyzed ethanol quantity does not therefore depend on D. However, the flow rate must be limited to permit the establishment of the equilibrium between the two phases.

With the apparatus and operating conditions described above, for a 10% v/v ethanol medium and with a constant temperature, we observe that the peak heights vary by less than 1% for a carrier gas flow rate of 200–1000 mL/min. For greater values of the flow rate, the heights begin to decrease slowly. The flow rate used for the following tests is 600 mL/min.

Calibration. At a fixed temperature, we determine the ethanol content of unknown solutions (water/ethanol) by using the stripping method. The ethanol content of these unknown samples is calculated with the relation

$$\%\ \text{ethanol (sample, v/v)} = \frac{\text{sample peak ht}}{\text{std peak ht}} \times \%\ \text{ethanol (std, v/v)}$$

The same chromatographic sensitivity is used for sample and standard solution. A solution containing 5.14% v/v ethanol in water is taken as a standard. In the range 4.19–7.96% a linear correlation exists between the sample peak height and its calculated ethanol content (% v/v) (Figure 3).

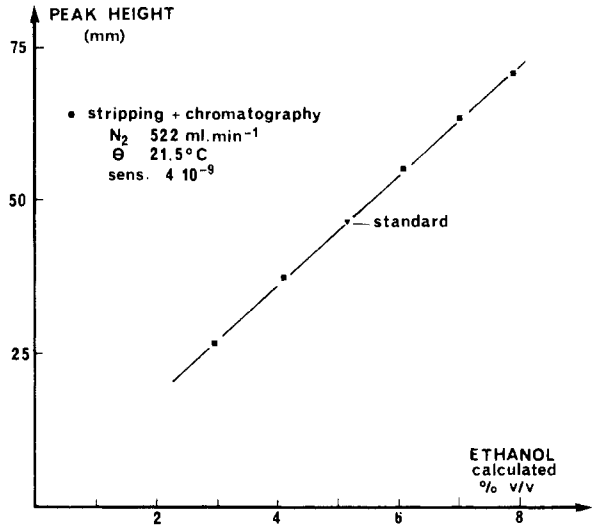

Figure 3. Stripping method calibration.

The ethanol values are compared with those given by the reference pycnometric method. We find a mean relative deviation between the two methods of 2%, the value by the stripping method being the lower (Table I).

The use of an internal standard requires the addition of a constituent showing a peak in the same conditions as ethanol (volatility, flame ionization detection, peak height). Instead we prefer to use a standard solution with an eth-

Table I. Determination of Ethanol in Water/Ethanol Solutions with the Stripping Method, Comparison with Pycnometry

Stripping + Chromatographic Analysis					
oper condtns	21.5 °C	$s = 4 \times 10^{-9}$		$D = 522$ mL/min	
peak ht, mm	37.0	46.5	55.0	63.4	70.4
calcd ethanol cont (% v/v)	4.09		6.08	7.01	7.78
Pycnometry					
ethanol cont (% v/v)	4.19	5.14 (std)	6.16	7.10	7.94
ethanol cont rel dev, %	2.4		1.3	1.3	2

anol content within ±1% of that of the sample. For on-line analysis in the plant, the apparatus should be calibrated periodically.

Tests with Complex Products. Beet wine consists of water and ethanol (5–9% v/v) and other substances (1%) such as particles in suspension (yeasts, proteins) or soluble particles (mineral salts, unfermented sugars, volatile constituents, ...).

The stripping method applied to beet wine is compared with classical analytical methods: ebulliometry (wine and its distillate), pycnometry, and areometry (distillate).

The experimental conditions for the stripping method are the same as those used previously for water/ethanol solutions (D = 600 mL/min, Θ = 20 °C). The peak heights vary by less than 1% in 1 h (12 peaks), the mean height being 200 mm.

We use a standard water/ethanol solution, with an ethanol content similar to that of the studied sample.

The results for the distillate, using the different methods are consistent; but, for the stripping method applied to the wine, we obtain higher values: +15%. This difference is attributed to the presence of organic and mineral salts, influencing the volatility of ethanol. It is necessary to have a standard solution more representative of the wine medium.

The addition of salts to an aqueous solution containing a volatile component (ethanol) modifies the liquid/vapor equilibrium. The salt ions create an electrostatic field attracting preferentially the more polarized water molecules. This involves a decrease in ethanol solubility in the solution or an increase in its activity coefficient. This effect depends on the quantity of salt in solution and on the difference in solubility of each pure component (Burns and Furter, 1979).

To the standard water/ethanol solution we add potassium sulfate, a mineral salt present in distillery products. The added quantity is equal to the ashes determined for wine, 1 g/100 mL. Potassium sulfate is insoluble in the more volatile component, ethanol; it is soluble in water, 120 g/L at 25 °C. With these conditions we predict for the standard solutions an increase in the volatility of ethanol. With a water/ethanol solution (7.17% v/v) we verify that chromatographic peak heights vary linearly with salt concentration in the range 5–60 g of K_2SO_4/L (Figure 4).

We use, therefore, a water/ethanol/salt solution as a standard for the stripping method. The final results are in good agreement with pycnometry (less than 2%); see Table II. The other methods compared with pycnometry are less accurate: −3% with areometry on the distillate; ±5% with ebulliometry.

To test the influence of the different substances present in the wine, we isolate them and prepare a standard solution containing water/ethanol/substances. The results

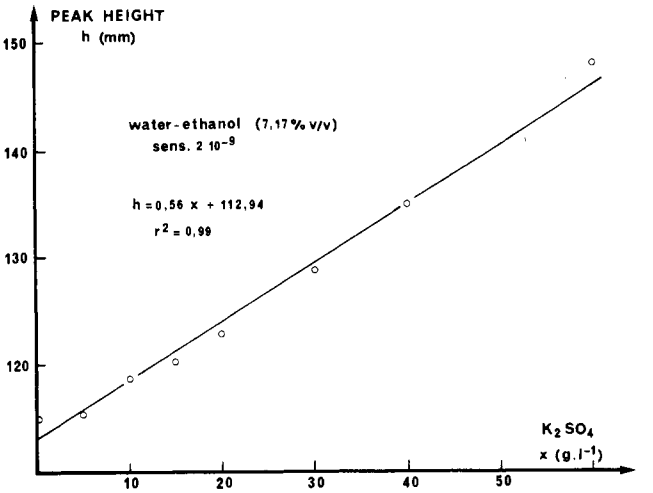

Figure 4. Influence of the salt content of the medium on ethanol stripping.

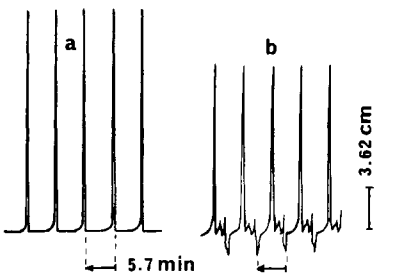

Figure 5. Chromatograms obtained after stripping of the ethanol present in products of beet distillation: (a) wine, 9% v/v ethanol (sensitivity 2×10^{-9}); (b) Vinasse, 0.01% v/v ethanol (sensitivity 2×10^{-12}).

Table II. Determination of Ethanol in a Beet Wine, Influence of Adding Salt to the Standard (K_2SO_4, 1 g/100 mL)

		stripping + chromatogr anal.				
pycnometry		peak ht, mm			% ethanol calcd for wine with	
std	distillate (from wine)	std	std + salt	wine	std	std + salt
8.05	7.34	129	135	120	7.74	7.39
7.19	7.29	124.5	133	137	7.91	7.40

are comparable to those obtained with a synthetic standard solution (water/ethanol/K_2SO_4).

In conclusion, for all distillery products, we propose to have a standard solution consisting of water, ethanol, and potassium sulfate.

The latter is added in the same proportion as the ashes content of the studied product.

With a complex medium, the presence of foam is avoided by the addition of an antifoaming agent: 0.2 mL/L of Bevaloid. It may already have been added during the fermentation process.

Tests on a wort during fermentation show no influence of carbon dioxide on the chromatographic ethanol determination.

In Nov 1983, tests were made with beet wine (9% v/v ethanol) and vinasse (0.01%) at the production plant of Cooperative Agricole de Distillation de la Brie in Provins, France (Figure 5). Ethanol contents have been calculated with both the heights and the areas of the peaks, with a difference of 2%.

The vinasse, issued from the distillation column, at 75 °C, must be cooled quickly during sampling (15 °C) to

avoid losses in the vapor phase by flash evaporation.

In view of the wide range of ethanol concentration in the distillery products, it seems important to use a modular sampling and chromatographic setup.

CONCLUSION

This study has enabled us to test a quick method (5 min) for evaluating the amount of ethanol in complex products. Losses per analysis are low. The results with a beet wine are comparable to the pycnometric reference laboratory method.

This technique has been tested with many products of grape or beet distillery. It may be applied to other ethanol-containing products, in other fields. By changing sensitivity, other volatile constituents may be analyzed. Automation is possible, and the apparatus can be implemented on derivation of the process for continuous analysis and control.

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Supercritical Methanol: An Efficacious Technique for the Extraction of Bound Pesticide Residues from Soil and Plant Samples

Peter Capriel,* Albert Haisch, and Shahamat U. Khan

Soil and plant samples containing bound ^{14}C residues of a number of pesticides and/or their metabolites were extracted with supercritical methanol. In a parallel experiment they were subjected to the high-temperature distillation technique. The extracts or the distillates were purified and analyzed by gas chromatography and gas chromatography–mass spectrometry. A comparison between the results obtained with both the techniques revealed that better recoveries of ^{14}C and higher concentrations of residues identified were obtained by the extraction with supercritical methanol. The work demonstrates the feasibility of supercritical fluid technique for the extraction of bound pesticide residues from soil and plants often not detectable in routine residue analysis.

INTRODUCTION

Studies using radioisotopes as tracers within pesticide molecules have revealed that a considerable portion of pesticide residues may remain bound (nonextractable) in soil and plants (Khan, 1982b; Huber and Otto, 1983). Bound residues in soil and plants are not generally detected in routine residue analysis. Thus, for a long time the possible soil or plant burden of total pesticide residues has been underestimated.

During the past few years determination of the nature and quantities of bound pesticide residues in soil and plants has been a challenging problem for a number of research workers. In most of the studies reported in the literature, quantification of ^{14}C-bound residues in soil or plants has been achieved by combustion. This method is limited to the determination of total ^{14}C bound residues and cannot be used to identify the chemical form of the bound residues. Attempts have also been made to extract and/or release bound pesticide residues by the milder to harsher methods. Drastic extractive procedures destroy the structure of soil or plants by solubilizing the materials, and strong acid or base hydrolysis often results in the destruction of the identity of bound residues (Khan, 1982b; Huber and Otto, 1983).

A pyrolysis technique for estimation of bound residues of chloroaniline compounds in plants was reported by Balba et al. (1979). Similar technique was developed by Khan and Hamilton (1980) involving high-temperature distillation (HTD) of the solvent-extracted soil or plant material to release bound ^{14}C residues. The released bound ^{14}C residues were collected in different solvents and analyzed by gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS). In recent years this technique has been widely used to determine the chemical identity of bound ^{14}C residues of pesticides and/or me-

Bayer. Landesanstalt fur Bodenkultur und Pflanzenbau, 8000 Munchen 19, Federal Republic of Germany (P.C., A.H.), and Chemistry and Biology Research Institute, Research Branch, Agriculture Canada, Ottawa, Ontario, Canada K1A 0C6 (S.U.K.).

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