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NEW METHOD FOR QUANTITATIVE ESSENTIAL OIL ANALYSIS

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SUMMARY

A new method for quantitative essential oil analysis, using combined steam distillation–extraction is described. The construction of the micro-version apparatus is such that all the volatile material is collected in only 1 ml of dichloromethane containing a suitable internal standard. The main advantage is that no further enrichment by evaporation is required. The method allows the quantitative analysis of total essential oil content and the percentage of the individual compounds in less than 4 h, including sample preparation, steam distillation–extraction and capillary gas chromatographic analysis.

INTRODUCTION

The objective of this work was to develop a new method for isolating essential oils in such a way that the total oil content as well as the individual components could be quantitatively evaluated. Following characteristics were deemed desirable for such a method: applicability when very low oil amounts are present and with only a small amount of material, speed compatible with routine measurement requirements, automatization potential and of course reliability.

The commonly used methods for estimating volatile organic material from natural sources are steam distillation, solvent extraction (Soxhlet), heat desorption or vapour collection by cryogenic concentration or by adsorption on solid adsorbents (charcoal, silica gel, Tenax GC). Steam distillation and solvent extraction as conventionally applied result in severe losses of volatile material because the solutions containing the oil must be concentrated by evaporation. Trace compounds even present in ultrapure solvents disturb the analysis when working in the ppm range. By solvent extraction non-volatile compounds are extracted as well. These can cause artefacts on evaporation–distillation. Heat desorption and vapour collection are unreproducible and prone to artefacts again, especially when working in the ppm range. Confronted with the problem of determining very low essential oil contents in flowers hardly smelling at all (see below) it seemed to us that the continuous steam-distillation with continuous extraction device described by Likens and Nickerson[1] looked most promising. This has however the disadvantage that evaporation of the solvent is required. The apparatus discussed in the present paper avoids this drawback and

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moreover uses the heavier than water solvent dichloromethane. Some examples, one with a very low oil content (*Rhododendron simsii ca.* 20 ppm) and two with a relative high oil content (*Humulus lupulus* or hops *ca.* 1 % and *Piper nigrum ca.* 3 %) are chosen to illustrate the new technique.

EXPERIMENTAL

Continuous steam distillation — continuous liquid–liquid extraction apparatus (Fig. 1)

The plant material (1 g of ground hops, 1 g of ground pepper, 15 g of cut-minced flowers, *e.g. R. simsii* flowers) is placed in a 50- or 100-ml flask (A) and distilled water is added so that the material is sufficiently wet:20 ml for hops, 10 ml for pepper, 30 ml for *R. simsii* flowers. A 1 ml volume of dichloromethane containing a suitable internal standard is introduced in B, having a content of 2 ml. Cleaned boiling chips are added to A and B. Before starting the procedure 1.5 ml of dichloromethane and 1.5 ml of water are introduced into C with a syringe via the open part of the system (H). This is the amount needed to fill the demixing–return arms (D and E). The vapour transport arms are thermally isolated. When the two-phase system has settled in the demixing part of the apparatus (C), the dichloromethane reflux is started by heating B in a water bath at 90°C. After 5 min steam is generated by applying heat to flask A with an oil bath at 140°C. These vapours are condensed in C by a cold finger in which ice water is circulated. The construction of the apparatus is such that the high-density layer (dichloromethane) returns through arm D in B; the low-density layer (water) through arm E in A.

After 1 h the steam distillation is stopped while the solvent extraction is continued for 20 min more. In this way all the volatile material is collected in *ca.* 1 ml of dichloromethane. During the extraction some dichloromethane from the demixing part may get into flask B or eventually a small amount of the solvent may be lost through evaporation. This does not matter with the internal standard used as described. Subsequently 1–3 μl of the solution are analysed as such by capillary gas chromatography.

Capillary gas chromatography

A Varian 3700 gas chromatograph equipped with a Varian CDS 111 electronic integrator was used in this study. The aroma standard mixtures and the extracts of the *R. simsii* flowers were analyzed on a 17 m × 0.25 mm I.D. capillary column roughened by whisker formation[2,3] deactivated with TIPA (tri-isopropanolamine)[3,4] and statically coated[5] with 3 mg Superox 20M (RSL, Eke, Belgium)[6] per ml of dichloromethane. The column was temperature programmed from 50°C to 200°C at 2°C min^{-1} with a hydrogen carrier gas flow-rate of 1 ml min^{-1}. The sensitivity setting of the instruments was 2 or $4 \cdot 10^{-11}$. The hop extracts were analyzed on a 45 m × 0.5 mm I.D. column statically coated with 3 mg Superox 0.1 per ml of dichloromethane. The column was temperature programmed from 70°C to 200°C at 2°C min^{-1} with a hydrogen carrier gas flow-rate of 5 ml min^{-1}.

Sample introduction was achieved with splitting (1 to 5–10) in a home-built all-glass split device filled with silanized glass wool. The hot needle injection technique[7] was applied by keeping the needle for 5 sec in the heated injector device before pushing the plunger. The outlet of the capillary column was introduced 2.5 cm in the

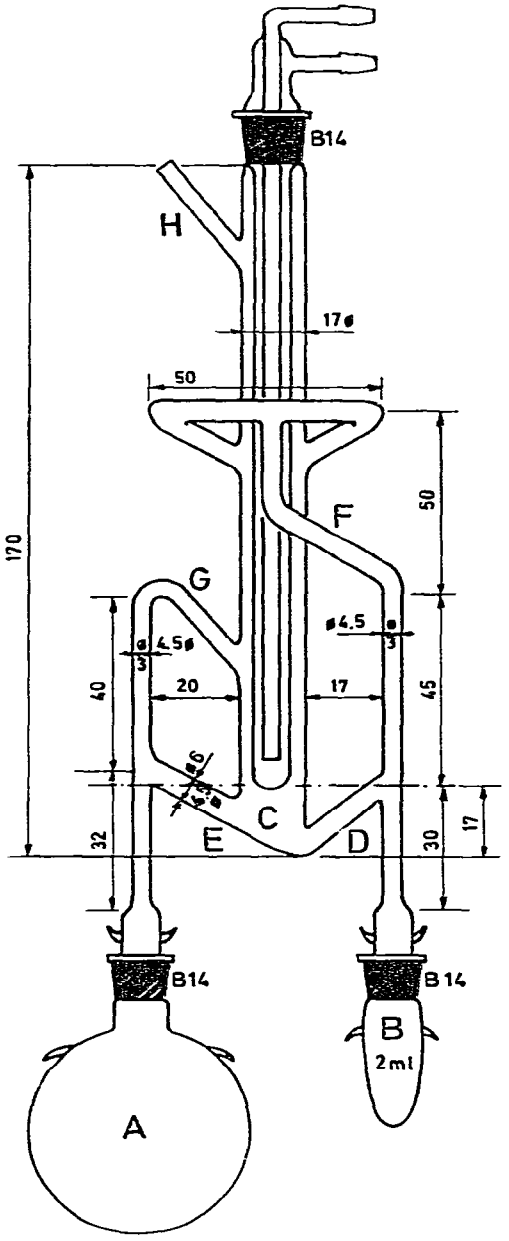

Fig. 1. Scale drawing in millimetres of the micro steam distillation–extraction apparatus (available from RSL, Eke, Belgium, and Alltech, Deerfield, IL, U.S.A.).

flame tip of the FID detector (Varian 3700) using in this way the burning gas as purge-gas.

For identification the above-mentioned columns were installed in a Finnigan Model 3200 quadrupole mass spectrometer equipped with a data system model 6000.

The capillary column was directly coupled to the ion source[8]. Spectra were recorded in the electron impact mode and chemical ionization mode using methane as reagent gas.

Odour evaluation was performed by splitting the capillary column outlet as described recently[9].

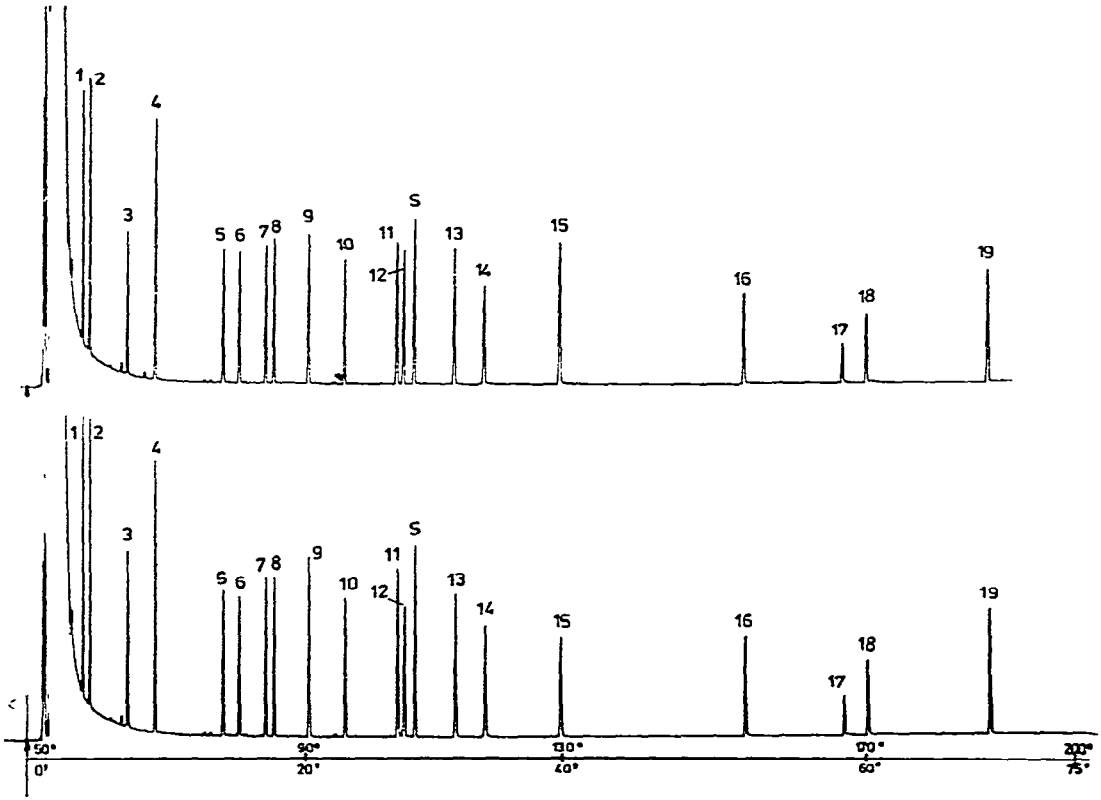

Fig. 2. Analysis of an aroma test mixture. A, Mixture as provided. B, Mixture after steam distillation–solvent extraction. Column, 17 m × 0.25 mm I.D. Whiskered, TIPA deactivated, statically coated with 0.3% Superox 20M. Temperature programmed 50°C to 200°C at 2°C min^{-1}. Hydrogen carrier gas flow-rate 1 ml min^{-1}. Amount per compound, 20 ng. For peaks, see Table I.

RESULTS AND DISCUSSION

Recovery

The quantitative aspect of the recovery was checked with the aroma test mixture of Fig. 2A. The mixture was dissolved in 1 ml of dichloromethane and 50 μg ethyl decanoate (S) was added as internal standard. Fig. 2B shows the analysis of the same amount of the mixture after steam distillation–extraction. Before starting the procedure 50 μg of internal standard was added to the 1 ml of extraction solvent. The total recovery and the recovery of the individual compounds are given in Table I.

TABLE I

RECOVERY OF THE METHOD FOR TEST COMPOUNDS

Peak No.	*Compound*	*Recovery (%)*	*Peak number*	*Compound*	*Recovery (%)*
1	Hexanal	102	11	Acetophenone	104
2	β-Pinene	97	12	Hexadecane	90
3	*trans*-2-Hexenal	113	S	Ethyl decanoate	—
4	*p*-Cymene	100	13	Carvone	103
5	*cis*-3-Hexenol	100	14	Methyl salicylate	96
6	*trans*-2-Hexenol	102	15	Benzyl alcohol	70
7	Ethyl octanoate	97	16	Eugenol	94
8	1-Octene-3-ol	101	17	Farnesol 1	102
9	Benzaldehyde	106	18	Farnesol 2	94
10	Linalool	99	19	Benzyl benzoate	90
				Total	95

These data were obtained after 1 h distillation–extraction and there is no advantage in proceeding for longer times. The high recovery of volatile compounds in this short time is ascribed to the continuous extraction, the extraction power of dichloromethane and to the small solvent amount (1 ml) used, requiring no further enrichment by evaporation. The dichloromethane solution is used as such for glass capillary gas chromatography. Likens and Nickerson[1] worked with water–pentane, while we use water–dichloromethane as a two-phase solvent system. This requires the configuration of the return arms shown in Fig. 1.

Carbon disulphide was also evaluated as extracting solvent, offering the advantage of low response to flame ionization detection. The extraction yield for alcohols was however low, *e.g.* 65% for *cis*-3-hexanol and 40% for benzyl alcohol. Carbon disulphide can however be applied to extract hydrocarbons, *e.g.* in waste water, and other pollution problems. By changing the level of the return arms, the apparatus can be modified to use low-density solvents such as *n*-pentane, benzene, etc.

That 1 h of distillation–extraction is sufficient is further illustrated in Fig. 3, which shows the yield of essential oil of pepper (*ca.* 3%), hops (*ca.* 1%) and *R. simsii* (*Azalea indica*) flowers (20 ppm) as a function of the distillation–extraction time. In the three cases the maximum yield is reached in 1 h. Note that for non-polar oils or oils in which mono- and sesquiterpenes are present in more than 80% (hop and pepper oil) the maximum yield is reached after 15 min. For polar oils or oils in which the oxygen compounds dominate, 1 h is needed to increase the recovery of some individual compounds *e.g.* alcohols and high-boiling polar compounds. The reproducibility for practical applications is discussed below.

Capillary gas chromatography

Superox capillary columns are particularly stable for temperature-programmed analyses. The chromatograms presented were recorded at high sensitivity (1–20 ng per peak) still showing practically stable baselines. The whisker Superox column described was used for more than 2 years without showing loss in efficiency. The inertness of the column is evident in Fig. 2. The column was deactivated with a deactivating agent (TIPA) containing N and OH as previously described[3,4]. If activity

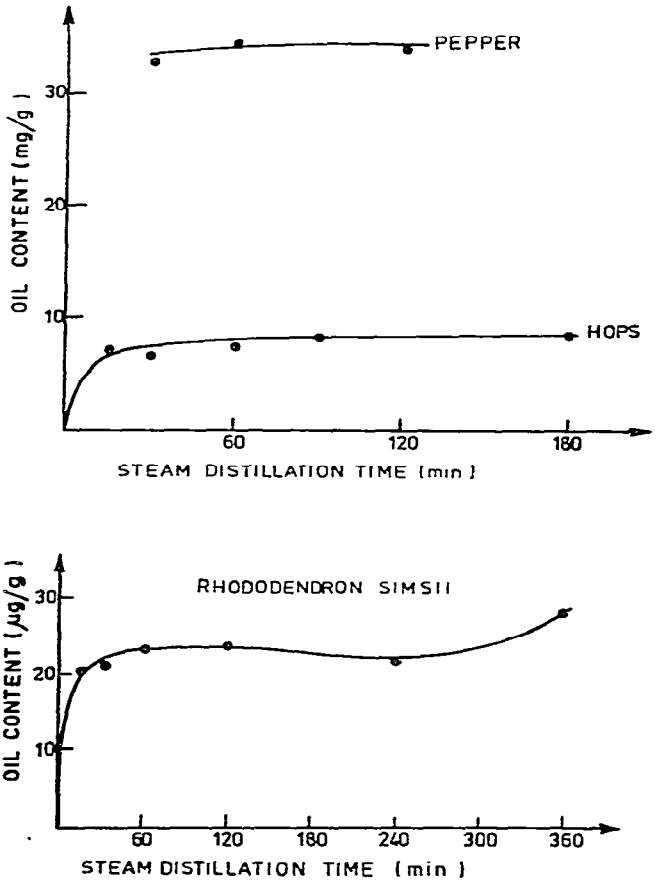

Fig. 3. Oil content of pepper, hops and *Rhododendron simsii* as a function of the steam distillation time.

is observed after long use, it can be suppressed by *in situ* deactivation with TIPA. This is routinely carried out in our laboratory by injecting three times 5 µl of a solution containing 5% tri-isopropanolamine in dichloromethane at 200°C column temperature. Due to the wide range of volatility of the compounds, the hot needle injection technique was adopted. The relative standard deviation for the aroma test mixture was 0.8% with the hot-needle injection technique and 2.1% with normal injection. The relative standard deviations per peak averaged 2.2% and 3.5%, respectively.

APPLICATIONS

Quantitative essential oil analysis applied to R. simsii (A. indica)

The *R. simsii* Planch (*A. indica* L.) is the most important ornamental plant in Belgium. Annual production is more than 28 million plants, of which more than 85% are exported.

By breeding, a typical Belgian Azalea collection was obtained over the years with a wide diversity of colour and flowering-times. The plants commercially cultivated

however have little or no smell. To make the plant even more popular, crossing techniques between the *R. simsii* and *R. scabrum* cultivars, having a good aroma but a very short flowering-period, are presently carried out. For the evaluation of the crossing results on the smell of the flowers a method was required for isolating the essential oil in such a way that the total oil content as well as the individual compounds could be quantitatively evaluated. The technique described above was successfully applied to different cultivars with essential oil amounts from 5 to 50 ppm. Some chromatograms are presented in Fig. 4. Fig. 4A shows the essential oil of the Osaka culti-

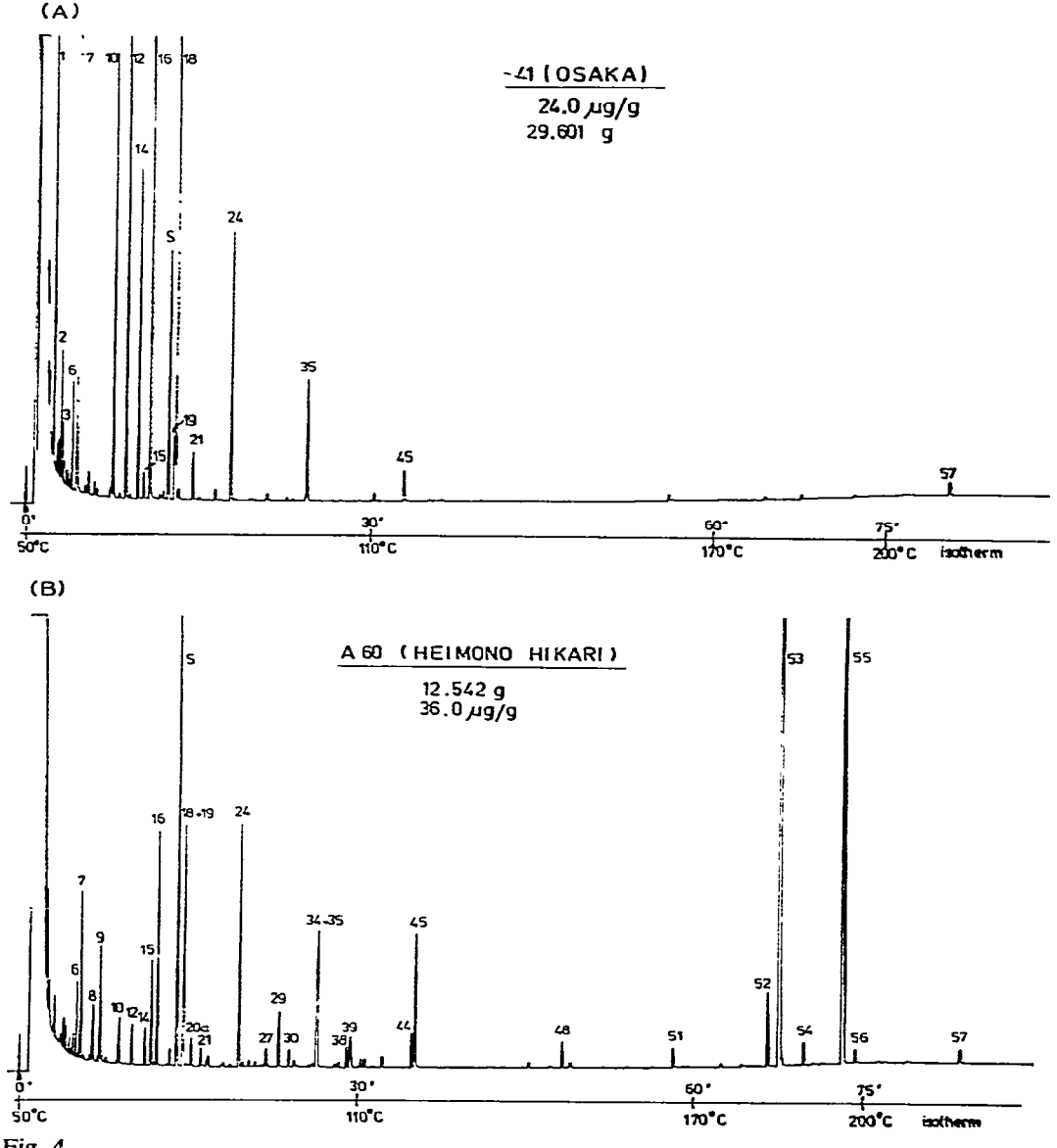

Fig. 4.

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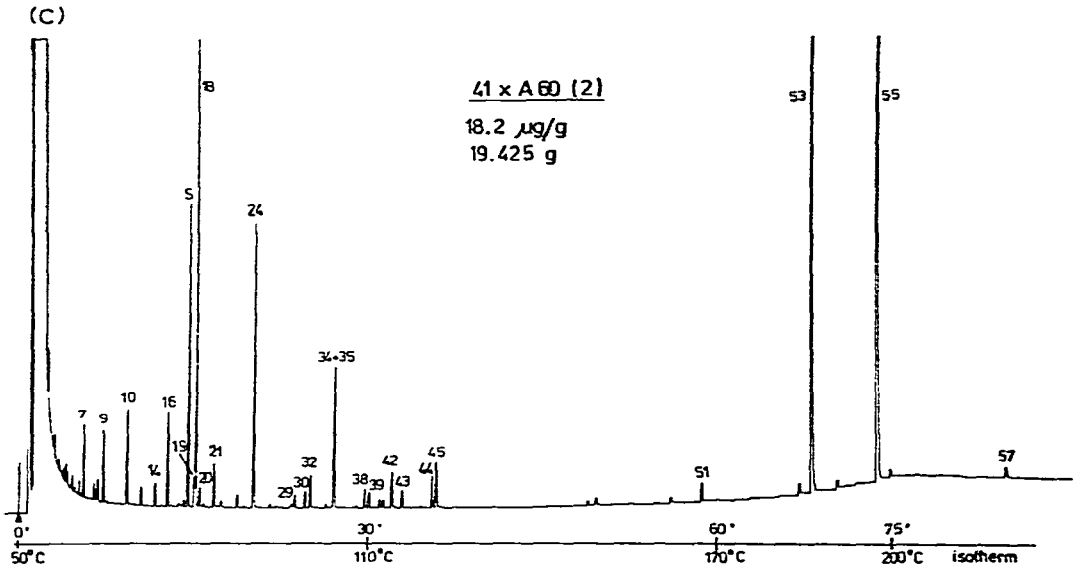

Fig. 4. (A) Essential oil of *Rhododendron simsii* (Osaka). (B) Essential oil of *R. scabrum* (Heimono Hikari). (C) Essential oil of a crossed cultivar. Chromatographic conditions as in Fig. 2. For peaks, see Table II.

var, a *R. simsii* having a very weak smell. The total essential oil amount calculated with ethyl octanoate as internal standard (S) is 24 ppm. The oil is completely dominated by highly volatile material and the concentration of compounds eluting after benzyl alcohol (peak 45) is very low. For the identity of the compounds see Table II. Fig. 4B shows the analysis of the Heimono Hikari variety, a *R. scabrum* having a good aroma but a very short flowering-period. In this case the highly volatile com-

TABLE II

COMPOUNDS IDENTIFIED IN *R. SIMSII*, *R. SCABRUM* AND CROSSED CULTIVARS

Peaks	*Compound*	*Peaks*	*Compound*
1	*n*-Hexanal	24	Linalool
6	Limonene	27	Methylbenzoate
7	*trans*-2-Hexenal	29	Acetophenone
8	3-Octanone	30	Sesquiterpene
9	3-Hydroxybutanone	32	Ethylbenzoate
10	2-Heptanol	35	α-Terpineol
12	*n*-Hexanol	38	Sesquiterpene
13	*trans*-3-Hexenol	39	Methyl salicylate
14	*cis*-3-Hexenol	42	Ethyl salicylate
15	Nonanal	43	α-Phenylethanol
16	*trans*-2-Hexenol	44	Geraniol
17 (S)	Ethyloctanoate	45	Benzyl alcohol
18	1-Octene-3-ol	47	*cis*-3-Hexenyl benzoate
20	Linalooloxide	48	*trans*-2-Hexenyl benzoate
21	Octadienol	53	Benzyl benzoate
		55	Benzyl salicylate

pounds are present in low concentration, whereas organoleptically interesting compounds such as benzyl benzoate (peak 53) and benzyl salicylate (peak 55) represent respectively 35.6% and 41.3% of the total oil content.

A cross between the Osaka and the Heimono Hikari variety resulted in a cultivar with a good aroma and an acceptable flowering-period. The improved smell is mainly due to the transfer of benzyl benzoate and benzyl salicylate from the *R. scabrum* plant in the cross (Fig. 4C). Organoleptic evaluation revealed that the compounds listed in Table III contribute to and/or characterize the aroma. By analyzing 22 cultivars, directives could be advanced to select a cross for large-scale cultivation. Further details will be published in an appropriate journal[10].

TABLE III

ODOUR EVALUATION OF THE RHODODENDRON CULTIVARS

+, ++, +++, ++++, increasing degree of contribution towards the aroma. ×, ××, ××××, increasing degree of contribution towards the characteristic typical flower smell.

Peaks	*Compounds*	*Contributing*	*Characteristic*
10	2-Heptanol	++	
18	1-Octene-3-ol	++	
24	Linalool	++++	
30	Sesquiterpene	++	
32	Ethyl benzoate	+	×
35	α-Terpineol	+	×
39	Methyl salicylate	+	
42	Ethyl salicylate	+++	××
43	α-Phenylethanol	+++	××
44	Geraniol	+++	××
53	Benzyl benzoate	++++	××××
55	Benzyl salicylate	++	××××

Quantitative essential oil analysis applied to hops

Aroma is an important quality criterion of hops. Hops with a pleasant aroma are considered superior in quality and it is assumed that the finer aroma is transferred to beer. Whether this is true or not is still unsettled. To contribute to this question, the volatile constituents from several varieties of hops harvested in 1978 have been investigated as a function of the picking time of the cones. The dried hop cones, stored at −4°C were ground just before analysis and 1 g was subjected to steam distillation–extraction as described. Fig. 5 shows the analysis of Record Hops containing 13.6 mg of essential oil per gram. Relative standard deviation of the whole procedure was calculated for five experiments and showed a value of 5.5%; the mean relative standard deviation per peak was 5.8%. The differences observed between the values of this practical application and the values obtained with the aroma standard mixture are due to the dependence of the standard deviation on the peak ratios. As could be expected the best values were obtained for equal peak heights[11]. The analysis of the high-quality Saaz Hop is shown in Fig. 6. This variety is distinguished among the examined varieties by its high content of β-farnesene (peak 8'). The influence of the harvest time on the oil content is illustrated in Fig. 7. The curves for Hallertau and Saaz hops level off at harvest time 3 while the oil content of the other hops still increased markedly. The practical value of these results for the brewing industry will be discussed elsewhere[12].

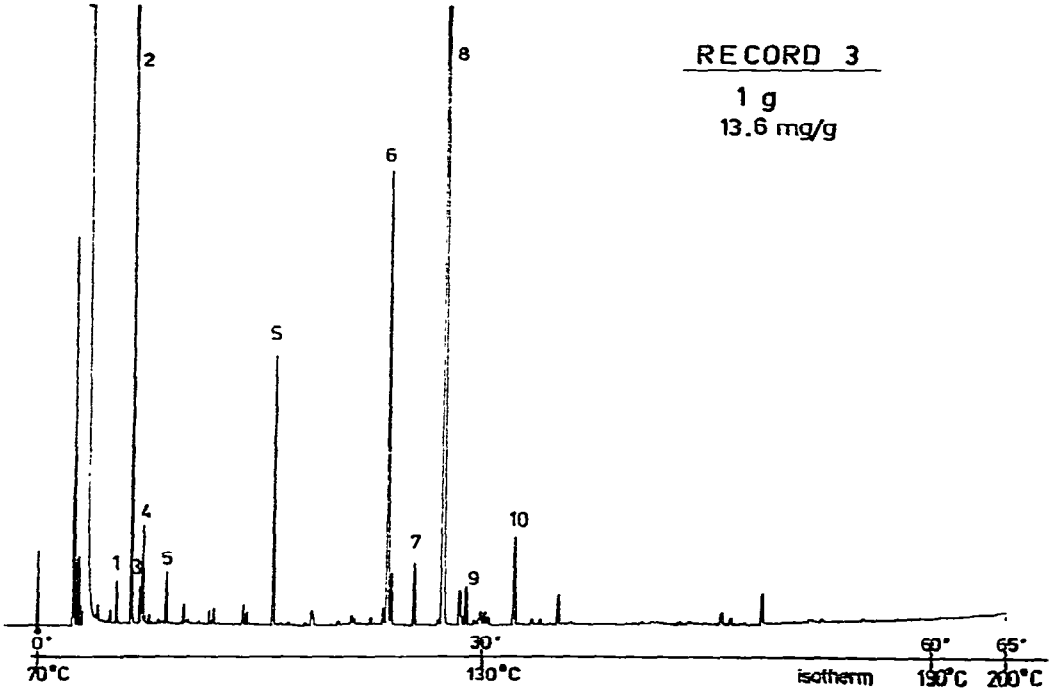

Fig. 5. Essential oil of Record Hops. Column, 45 m × 0.5 mm I.D. statically coated with 0.3% Superox 0.1. Temperature programmed 70°C to 200°C at 2°C min^{-1}. Hydrogen carrier gas flow-rate, 5 ml min^{-1}. Compounds: 1 = β-pinene; 2 = myrcene; 3 = 2-methylbutyl propionate; 4 = ester; 5 = ocimene; 6 = β-caryophyllene; 7 = methyl dec-4-enoate; 8 = humulene; 9 = methyldeca-4,8-dienoate; 10 = sesquiterpene.

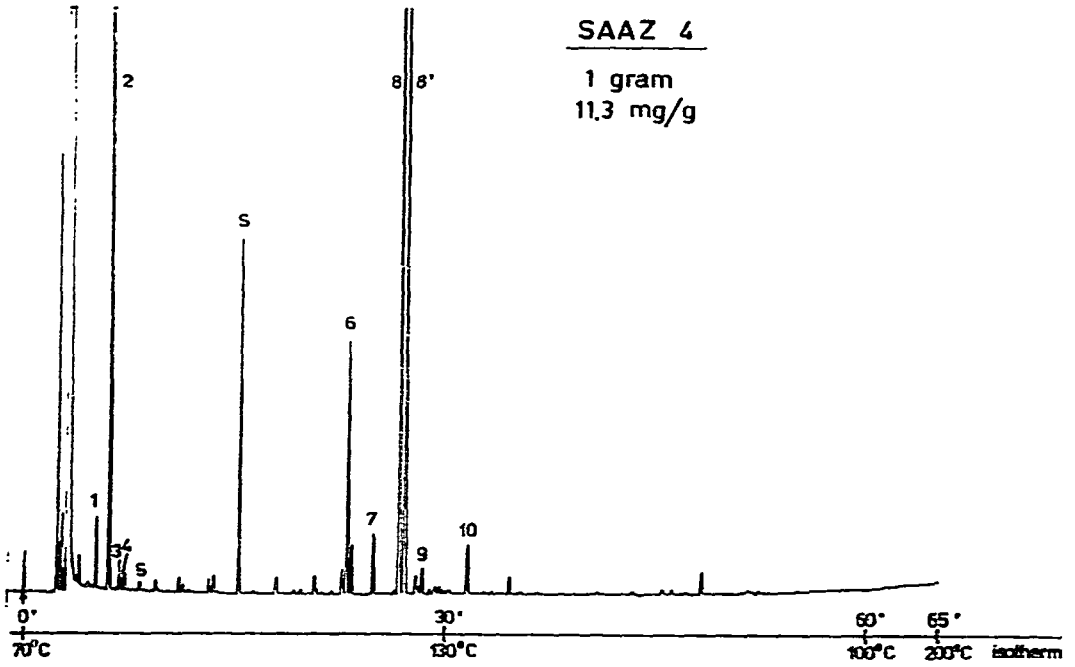

Fig. 6. Essential oil of Saaz Hops. Chromatographic conditions and compounds as for Fig. 5; peak 8′ = β-farnesene.

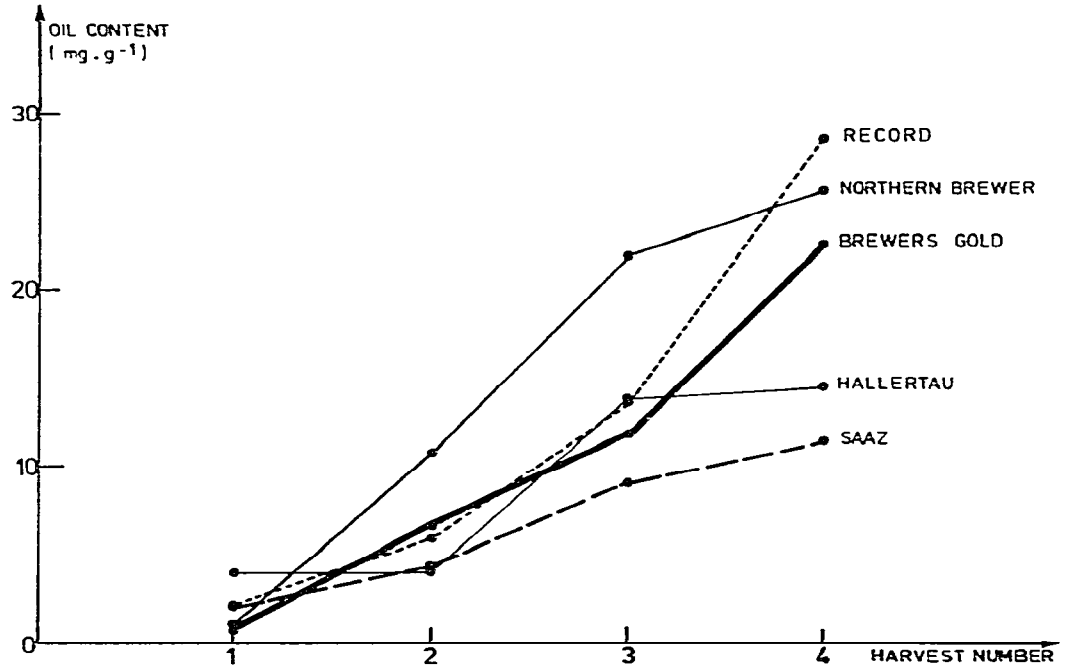

Fig. 7. Hop oil content of different varieties as a function of the harvest period spread over *ca.* 6 weeks: 1 = too early; 4 = too late.

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REFERENCES

1 S. Likens and G. Nickerson, *Proc. Amer. Soc. Brew. Chem.*, 1964, 5.
2 J. D. Schieke, N. R. Comins and V. Pretorius, *J. Chromatogr.*, 112 (1975) 97.
3 P. Sandra, M. Verstappe and M. Verzele, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 1 (1978) 28.
4 P. Sandra and M. Verzele, *Chromatographia*, 10 (1977) 419.
5 J. Bouche and M. Verzele, *J. Gas Chromatogr.*, 6 (1968) 501.
6 P. Sandra, M. Verzele, M. Verstappe and J. Verzele, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 2 (1979) 288.
7 K. Grob Jr. and H. P. Neukom, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 2 (1979) 15.
8 F. Vangaever, P. Sandra and M. Verzele, *Chromatographia*, 12 (1979) 153.
9 P. Sandra, T. Saeed, G. Redant, M. Godefroot, M. Verstappe and M. Verzele, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 3 (1980) 107.
10 M. Godefroot, P. Sandra, M. Verzele and J. Heursel, in preparation.
11 M. Godefroot, P. Sandra and M. Verzele, in preparation.
12 M. Verzele, M. Godefroot and P. Sandra, unpublished results.