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Solid-phase microextraction of hop volatiles Potential use for determination and verification of hop varieties ☆

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Abstract

The composition of hop essential oil is an important tool for evaluation of hop quality. As each hop variety has a typical essential oil pattern (fingerprint), hop oil analyses can be used to distinguish between hop varieties. The headspace solid-phase microextraction (SPME) method as described in this contribution is a simple sample preparation technique and represents an alternative procedure for essential oil fingerprint determination. Different SPME parameters (extraction temperature, extraction time and sample mass) were studied and the results were compared with those obtained by the routine distillation method. It is shown that SPME results can be used for determination and verification of varieties grown in Slovenia by means of principal components analysis.

Keywords: Hop; Essential oils; Headspace analysis; Prinicple components analysis; *Humulus lupulus*

1. Introduction

Hops, cones of the hop plant (*Humulus lupulus* L.), are used in brewing to add bitterness and aroma to beer. The quality of the final product — beer — depends on the hop variety used in the process. Those breweries that still use traditionally aromatic hops, tend to buy hops of known variety and origin. As each hop variety has a typical essential oil pattern (the so-called fingerprint of the variety), hop oil analyses are used for identification of hop varieties and determination of aroma properties [1–10].

Dry hops contains 0.5–2% of essential oil. It contains mainly terpene hydrocarbons and their oxidation products. About 400 peaks can be registered in capillary GC chromatograms and more than 200 components have been identified. The most important constituents are the monoterpene myrcene and the sesquiterpenes α-humulene and β-caryophyllene, which together represent about 80–90% of the total [11]. The routine method for hop essential oil analysis is based on oil isolation by steam distillation followed by gas chromatographic separation [12]. This requires a relatively large amount of sample (50–100 g) and is a time consuming procedure (3–4 h of distillation). The essential

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oil obtained in this way is diluted and injected into a GC system. The relative areas of the chromatographic peaks for the components of the essential oil are used as a rough approximation to the mass fraction of each component.

Solid-phase microextraction (SPME) is a relatively new sampling and concentration technique [13]. A sampling device — a fused silica fibre coated with a polymer sorbent — concentrates analytes by adsorption and/or absorption [14] and the extracted material is released into a GC system by means of thermal desorption in a GC injector. In the case of volatile analytes it can be used as a solvent-free headspace sampling technique [15]. Such a method is necessary when analyzing solid samples such as hop cones. Unfortunately, because of strong analyte–matrix interactions, it has limited quantification capabilities [16]. The limiting factor in mass transfer from the solid sample via the headspace to the fibre is the slow evaporation of high molecular weight volatiles, which increases the extraction time [17]. Sampling time could be shortened by increasing the extraction temperature, thus promoting the evaporation rate [18]. Despite this limitation, headspace SPME has been used as a tool for hop characterisation by monitoring the ratios between two important constituents (the sesquiterpenes α-humulene and β-caryophyllene) [19].

In this contribution, different SPME parameters (extraction temperature, extraction time and sample weight) were studied and results were compared with those obtained by the routine distillation method. The optimal SPME parameters were chosen considering the time of analysis and comparability of the results with those of the distillation method. Samples of the most important hop varieties grown in Slovenia were then analyzed by the optimized method, the results were processed by principal components analysis (PCA) and used to determine and verify hop varieties.

2. Experimental

2.1. Materials

A sample of the Aurora hop variety obtained from the world hop collection at the Institute of Hop Research and Brewing in Žalec (Slovenia) was used for method optimisation. The method was tested on 31 hop samples belonging to the four most important hop varieties grown in Slovenia: Aurora (nine samples), Celeia (eight samples), Magnum (six samples) and Savinjski golding (eight samples). They originate from different Slovenian hop growing areas, are representative of the 1999 and 2000 crops and were collected with the help of the Slovenian Agricultural Advisory Service (Žalec, Slovenia). All samples were air dried at 65°C and contained from 9 to 11% of moisture.

2.2. Sample preparation and analysis

Samples of hop essential oil were isolated by steam distillation using a distillation apparatus of Clavenger type [12]: 50 g of pulverized hop cones (ground in a coffee mill) were weighed into a 2000-ml distillation flask, 1000 ml of deionised water were added and the mixture was distilled for 4 h. Oils were collected from the condenser and 0.2 ml of oil was diluted with 5 ml of *n*-hexane. The components were separated by GC analysis on a Hewlett-Packard 5890 gas chromatograph (Palo Alto, CA, USA): 1.3–1.5 μl of sample were injected using flow splitting of 1:50, at 0.5 ml/min carrier gas-flow (N_2, 5.0), a HP-1 capillary column (Palo Alto, CA, USA) (25 m×0.2 mm, 0.11 μm) and a flame ionisation detector. The temperatures of injector and detector were 180 and 280°C, respectively. The temperature programme was 3 min at 60°C, from 60 to 190°C at a rate of 2.5°C/min and 1 min at 190°C. The chromatograms were recorded and integrated using a Hewlett Packard 3396A integrator. The results of the chromatography were given as relative areas of all essential oil components.

SPME sample preparation was as follows: hop cones were ground in a coffee mill. Various defined sample amounts were weighed into 40-ml headspace vials (0.05, 0.1, 0.25, 0.5, 1, 2, 4 and 6 g), closed with screw caps and stored in a freezer at −10°C until analyzed to prevent possible oxidation. The headspace vial was conditioned in a thermostatic water bath at the extraction temperature for 45 min, since this is the time required for the sample to reach the working temperature. The next step was SPME

extraction using a fibre with a 100-μm polydimethylsiloxane coating (Supelco, Bellefonte, PA, USA) at different extraction temperatures (26, 50, 60, 70 and 80°C). Extraction completed, the fibre was retracted and injected into the Hewlett-Packard 5890 gas chromatograph under the same conditions as described for the essential oil analysis, with the exception of a higher injection temperature (240°C). The split mode of injection was used as recommended [19].

2.3. Chemometric processing

Eleven chromatographic peaks were selected for further chemometric processing. For each sample, the sum of the areas of the 11 peaks in question was calculated. Indexes for each peak were defined as the quotient between the peak area for that peak and the sum of all 11 peaks in that sample. Samples described by such indexes were processed using PCA and a two-dimensional scatterplot was charted. The PCA chemometric step was performed using Statgraphics Plus for Windows 4.0 statistical software (Manugistic, Rockville, MD, USA).

2.4. Identification of essential oil components used in PCA

As well as myrcene and α-humulene, whenever possible, the compounds selected for PCA were identified by comparing retention times and MS spectra of the pure compounds: linalool (99%, Janssen Chimica), methyl octanoate (puriss. grade, Fluka, Dissenhofen, DE), methyl nonanoate (Dragoco, Holzminden, DE), 2-undecanone (98%, Merck, Darmstadt, DE), α-humulene (purum grade, Fluka, Dissenhofen, DE) and myrcene (technical grade, Ega-Chemie, DE). Identification of selected essential oil components was performed on a Varian GC/MS system (Walnut Creek, CA, USA) (STAR 3400 CX gas chromatograph coupled with a Saturn 2000 ion trap mass spectrometer). A sample of essential oil was prepared in the same way as described for the essential oil analysis. GC–MS chromatograms were obtained by the split injection mode (1:30); 1 μl of essential oil solution being injected at an injector temperature of 180°C on a RTX-5MS capillary column (Restek, Bellefonte, PA, USA) (30 m×0.25 mm, 0.25 μm), the carrier gas being helium 5.0 with a flow-rate of 1.5 ml/min at 60°C and the temperature programme being 3 min at 60°C and from 60 to 150°C at a rate of 1°C/min. Ion trap detection was carried out using electronic and chemical ionisation. Electron impact ionisation (EI) mass spectra were acquired under the following conditions: ion trap temperature: 180°C, ionisation energy: 70 eV, scan range: 50–400 m/z, multiplier: 2450 V and AGC target value of 32 300. Recorded EI mass spectra were compared to library spectra [20,21]. Molecular masses were determined by chemical ionisation using methanol as a reagent gas at an ion trap temperature of 180°C, scan range of 60–249 m/z, multiplier at 2450 V and an ARC target value of 5000.

Kovats retention indexes were determined on both GC systems using *n*-alkanes from C_{10} to C_{16}. For means of identification, the essential oil was fractionated into a hydrocarbon fraction and an oxygenated fraction using solid-phase extraction, a method described by Antonelli and Fabri [22]. A 500 mg/3 ml silica cartridge Bond Elut SI (Varian, Harbor City, CA, USA) was conditioned with 3 ml of *n*-pentane, 20 μl of the essential oil was loaded and the hydrocarbon fraction was eluted with 3 ml of *n*-pentane. The oxygenated fraction was eluted with 3 ml of anhydrous diethyl ether. The volumes of both fractions were reduced to 0.5 ml by evaporation at room temperature and injected into both GC systems.

3. Results and discussion

3.1. Optimisation of SPME method

Initial experiments were conducted to verify the sampling conditions in the reference describing SPME hop analysis [19]. The conditions described were as follows: fibre type: 100-μm polydimethylsiloxane, extraction temperature: 50°C, extraction time: 4 h and split mode of injection. The chromatograms obtained were to a great extent similar to the chromatograms obtained in the analysis of essential oil solution — minus the solvent peak. However, an absorption time of 4 h was not acceptable, since it prolongs total analysis time beyond practical limits.

3.1.1. The effects of extraction time and extraction temperature

Further trials were performed to shorten extraction time by a higher extraction temperature, which should accelerate mass transfer flux through the sampling system. A sample of Aurora hops was analyzed by the routine distillation method and by the SPME method at different extraction temperatures using 4 g of sample and an extraction time of 1 h. In all cases, the sum of all the essential oil peaks in the chromatogram varied only for ±10%, i.e., it was practically constant, which means that the fibre was saturated with hop volatiles regardless of the extraction temperature. Any changes in the composition of the absorbed phase were due to competition between different volatiles with different affinities towards the fibre. This is why we did not have to monitor the mass of the selected compounds absorbed on the fibre, and could use the relative areas of selected peaks in the chromatogram. The relative areas of two major components (more volatile monoterpene myrcene and less volatile sesquiterpene α-humulene) in the hop oil chromatogram were compared, and the results are presented in Fig. 1. The fibre concentration of myrcene decreases as the extraction temperature increases. The contrary is observed for α-humulene. The relative area of its chromatographic peak increases with increasing extraction temperature and tends to reach the value obtained for this compound by direct injection of the solution of the essential oil. However, even higher extraction temperatures were not used in the analysis because of the danger of oil oxidation [19] and the practical limits of the water bath.

For further optimisation, two extraction temperatures (60 and 70°C) were chosen and used when optimizing the extraction time. Again, 4 g of Aurora hops were analyzed at both extraction temperatures and extraction times from 15 min to 4 h. The sum of all the essential oil peaks in the chromatogram again remained constant regardless of different SPME conditions. The values measured for volatiles myrcene and α-humulene were compared to those obtained by gas chromatography of the distilled essential oil. Myrcene exhibits smaller fibre concentrations at longer extraction times and α-humulene exhibits higher fibre concentrations at longer extraction times.

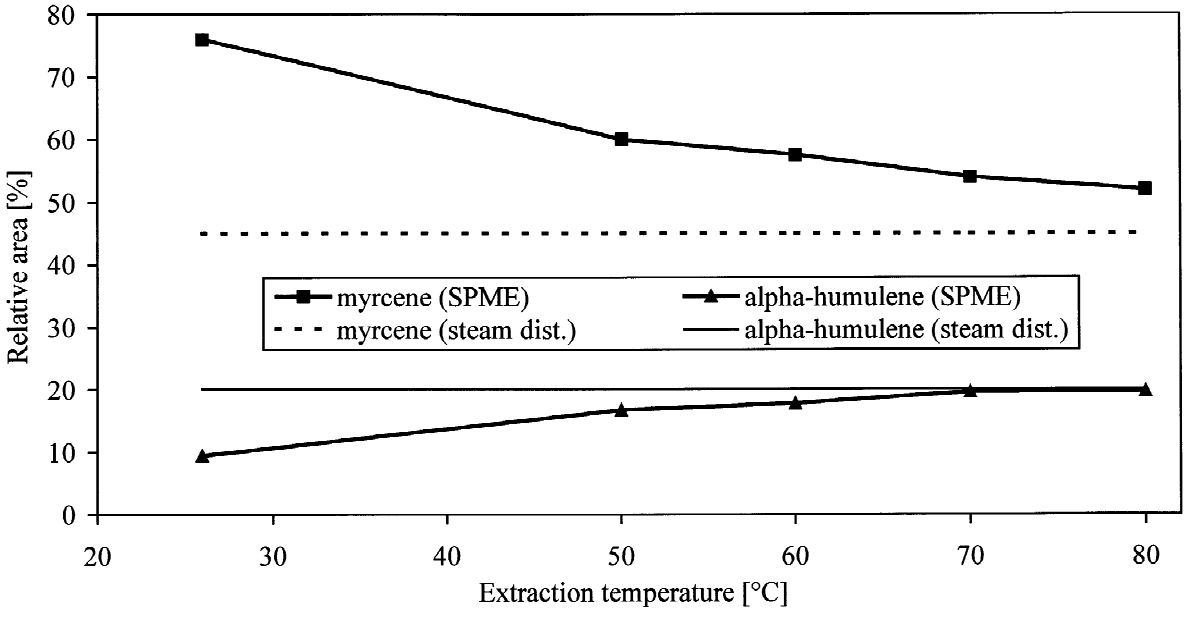

Fig. 1. Headspace SPME–GC analysis of hop volatiles. Relative areas for myrcene and α-humulene at different extraction temperatures compared with relative areas for the same compounds in essential oil obtained by routine steam distillation (Section 2.2.). Extraction time = 60 min, sample weight = 4 g.

It is clear that with higher extraction temperatures and longer extraction times the fibre concentrations of less volatile compounds increase, while those of more volatile compounds decrease. Therefore, it seems plausible, that the evaporation rate is the limiting factor in the mass transfer from the solid sample to the gaseous phase [17]. Because the system, even after 4 h, does not reach equilibrium, it is necessary to choose a non-equilibrium extraction time for routine work. Here the most important criterion is the similarity of the composition of the extracted volatiles and the composition of the actual essential oil. It is clear that it is impossible to choose SPME parameters in such a way that this method would give results identical to GC analysis of the essential oil for all oil constituents. Since a short time of analysis is desired, an extraction of 30 min was chosen and, consequently, a temperature of 70°C was selected to ensure faster evaporation from the solid sample. A 30-min extraction is a compromise which in practice does not prolong the time of the analysis, since chromatography itself takes 60 min. In the future, it could even give a possibility of shortening the time of the entire analysis, if we shorten the time of chromatography. Using these parameters in sample preparation we have obtained chromatograms that in general exhibit peaks of higher intensities for more volatile compounds and peaks of lower intensities for less volatile compounds if compared to the chromatograms of the essential oil. Additionally, compared to the chromatograms of the essential oil, SPME chromatograms show fewer very small peaks with relative areas less than 0.1%. At the same time we did not notice any increase in the content of oxidation products like humulene- and caryophyllene epoxides.

3.1.2. The effect of sample weight

The influence of sample weight on the composition of the extracted volatiles was also studied, using 30 min of extraction at 70°C. The fibre concentration of myrcene increases with increasing sample mass, and the fibre concentration of α-humulene decreases (Fig. 2). However, the total area of all the essential oil peaks in the chromatogram was again practically constant. Such results clearly indicate that fibre was already saturated with hop volatiles at the lowest amount of sample tested, and consequently, any

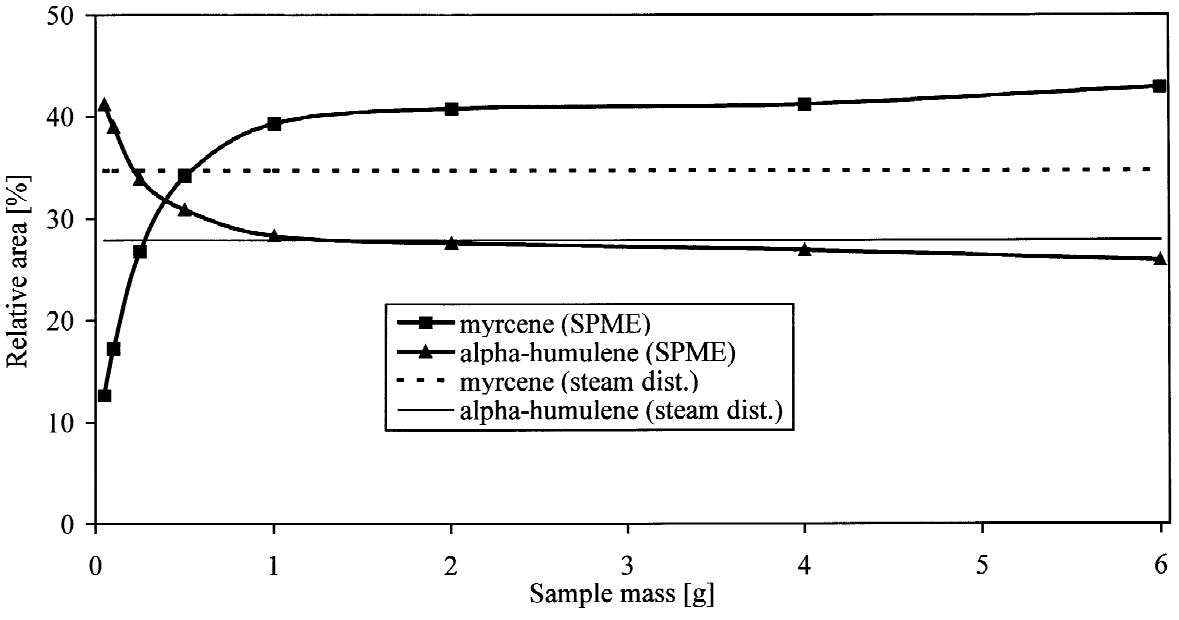

Fig. 2. Headspace SPME–GC analysis of hop volatiles. Relative areas for myrcene and α-humulene for different amounts of sample compared with relative areas for the same compounds in essential oil obtained by routine steam distillation (Section 2.2.). Extraction time = 30 min, extraction temperature = 70°C.

changes in the composition of the absorbed phase are due to competition between different volatiles exhibiting different affinities towards the fibre. It is known that the fibre promotes the absorption of less volatile compounds such as α-humulene and discourages absorption of more volatile compounds such as myrcene [23]. The driving force promoting a high myrcene fibre concentration is therefore its high concentration in the headspace, which is further supported by the large excess of essential oil in sample quantities over 1 g. According to our estimate, approximately 30–50 mg of hops already contain the same amount of essential oil as that extracted by the fibre and then injected into the GC system. In this case, 1 g of hops means a 20–30 fold excess of the essential oil and, consequently, the composition and quantity of the hop volatiles in the sample used for extraction do not change significantly during the SPME sampling. When dealing with smaller sample weights, this is not the case and extraction from such a small sample significantly decreases the total amount of myrcene in the sample used for extraction and consequently decreases its headspace concentration. Since the fibre has a much higher affinity towards α-humulene, it requires a much lower headspace concentration of this component for a certain fibre concentration to be obtained. The extraction of α-humulene is therefore not so sensitive to smaller sample weights and when very small samples are used a competitive replacement of myrcene by α-humulene takes place. The changes are less pronounced if the sample weight exceeds 2 g. As reproducible results were desired, such an amount of sample was chosen and used for routine analysis.

3.1.3. Repeatability

The repeatability of the method performed under optimized conditions was determined by analyzing six subsamples of the above-mentioned Aurora sample using an extraction temperature of 70°C, an extraction time of 30 min and a sample weight of 2 g. The relative standard deviations of relative peak areas exceeding 1% ranged from ±4 to ±6%, and for the peaks in the range between 0.1 and 1% from ±7 to ±10%. A typical chromatogram is shown in Fig. 3.

3.2. Determination and verification of hop varieties

For fingerprinting, 31 samples of the 1999 and

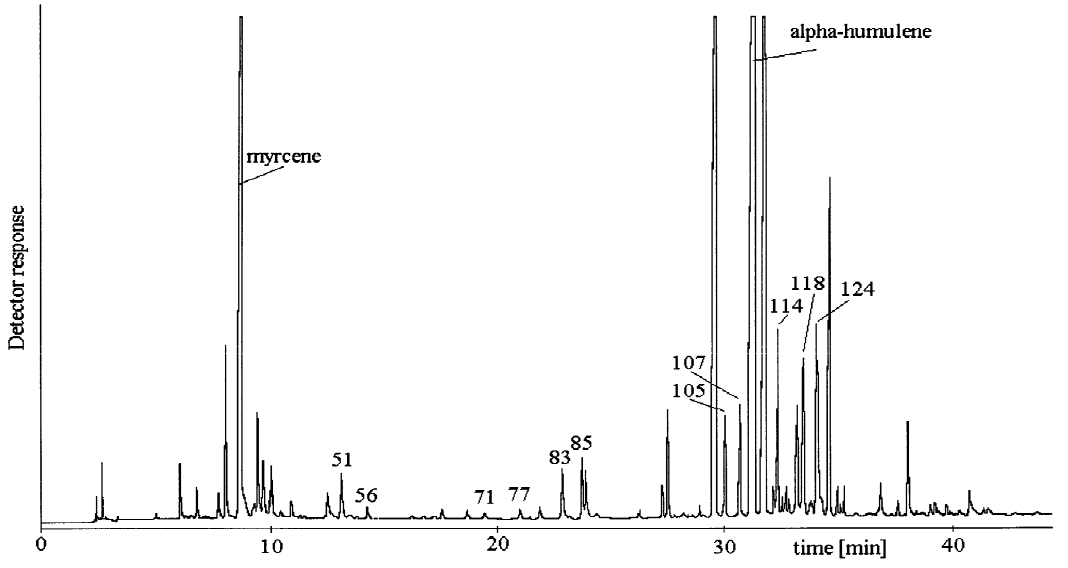

Fig. 3. A typical headspace SPME gas chromatogram of an Aurora hop sample. Extraction conditions: extraction temperature = 70°C, extraction time = 30 min, sample weight = 2 g. For GC conditions see Section 2.2, for details on peaks identities see Table 1.

Table 1
Chromatographic peaks used in chemometric analysis

Peak no.	Component name	R_I[a]	R_I[b]
51	Linalool	1084	1100
56	Methyl octanoate	1109	1125
71	Methyl nonanoate	1209	1226
77	N.i.[c] oxygenated compound (170 amu)	1238	1258
83	2-Undecanone	1274	1295
85	Methyl-deca-4-enoate (*trans*)	1291	1311
105	N.i.[c] sesquiterpene	1412	1418
107	α-Bergamotene	1428	1430
114	γ-Muurolene	1459	1468
118	α-Selinene	1476	1483
124	γ-Cadinene	1493	1503

[a] Retention Index on HP-1 column.
[b] Retention Index on RTX-5 column.
[c] Not identified.

2000 hop crops representing the most common and commercially most important hop varieties grown in Slovenia (Aurora, Celeia, Savinjski golding and Magnum) were analyzed by the described SPME GC method, consequently 11 characteristic peaks/compounds were selected from the GC chromatograms and used for chemometric analysis (Table 1). When standards of suitable pure compounds were obtainable, the essential oil constituents were identified on the basis of their retention times, mass spectra and coinjection of samples and standards (constituents giving the peaks 51, 56, 71 and 83). Compounds giving the peaks 85, 107, 114 and 124 were identified on the basis of EI and chemical ionization (CI) mass spectra, Kovats retention indexes and comparison with literature data [2,3]. The compound giving peak 118 was identified by analyzing a SPME sample obtained by mixing a hop sample and a celery sample, followed by mass spectral identification [24]. In the case of the unidentified compounds giving peaks 77 and 105, their retention indexes and mass spectra are presented (Table 1 and Figs. 4 and 5).

Data were processed with Statgraphic plus software using principal components analysis. As seen from the two-dimensional scatterplot obtained (Fig. 6), which accounts for 76% of the variability of the original data, this technique can be used to dis-

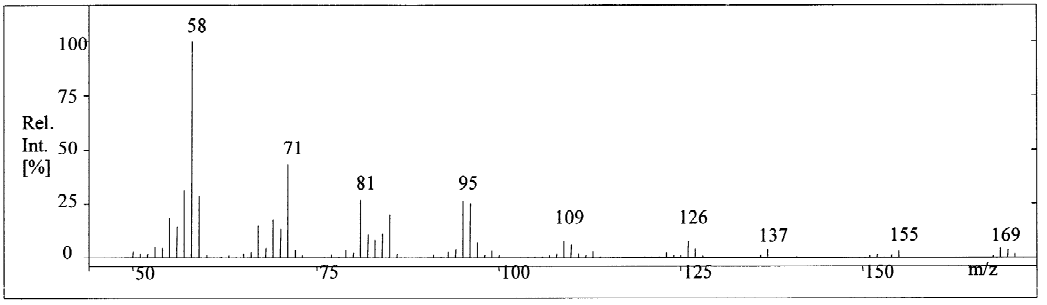

Fig. 4. EI mass spectrum of the compound giving chromatographic peak 77. For experimental details see Section 2.2.

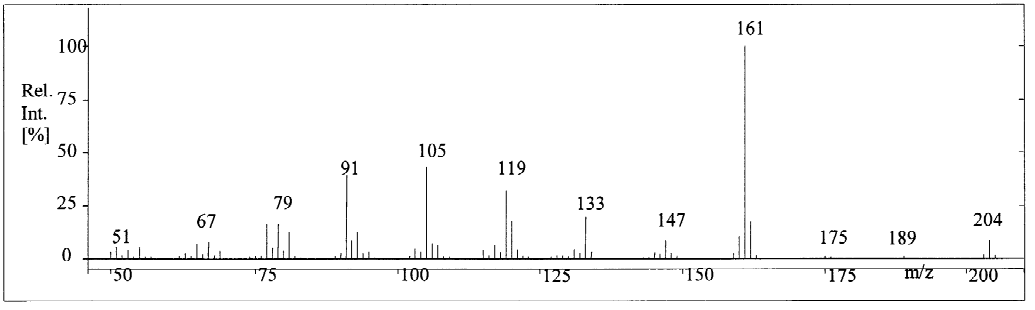

Fig. 5. EI mass spectrum of the compound giving chromatographic peak 105. For experimental conditions see Section 2.2.

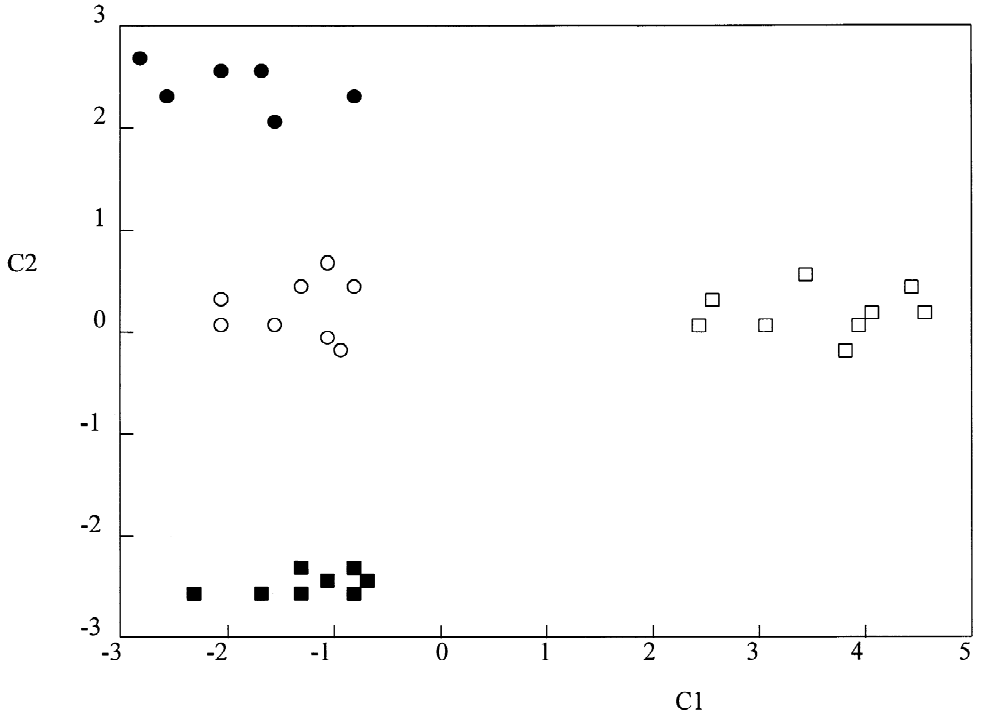

Fig. 6. Principal components analysis of essential oil components (C1 = component 1; C2 = component 2) performed on 31 hop samples of hop varieties grown in Slovenia: Aurora (□), Celeia (■), Magnum (●) and Savinjski golding (○).

tinguish between different hop varieties. The ability to distinguish depends of course on the group of hop varieties analyzed. In the case of our laboratory, where only hops from Slovenia were analyzed for this study and from the practical point of view (only Aurora, Celeia, Savinjski golding and Magnum are common and commercially important varieties in Slovenia) the system works very well. Since the composition of the essential oil exhibits some minor changes from year to year, the data base should contain data from several years. Presently, only the 1999 and the 2000 data are available and we intend to repeat the sampling in forthcoming years. Despite the usefulness of the system described, it has to be emphasized that on the scale of world hop production, where numerous varieties with minor differences are grown, such a system could lack the necessary resolution. But nevertheless, the problem of determing the varieties is rarely so complex, since we usually know what we are looking for and have some reference samples to work with and, consequently, the system described represents a handy and, in most practical cases, reliable tool for determination and verification of hop varieties.

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References

[1] C.P. Green, J. Inst. Brew. 103 (1997) 293.
[2] P. Perpete, L. Melotte, S. Dupire, S. Collin, J. Am. Soc. Brew. Chem. 56 (1998) 104.
[3] R.G. Buttery, L.C. Ling, J. Agric. Food Chem. 15 (1967) 531.

[4] S.T. Kenny, J. Am. Soc. Brew. Chem. 48 (1990) 3.
[5] D. Kralj, J. Zupanec, D. Vasilj, S. Kralj, J. Pšeničnik, J. Inst. Brew. 97 (1991) 197.
[6] M. Kač, M. Kovačevič, Monatsschr. Brauwiss. 53 (2000) 180.
[7] J. Freundorfer, J. Maier, L. Reiner, Monatsschr. Brauwiss. 44 (1991) 176.
[8] L.E. Stenroos, K.J. Siebert, J. Am. Soc. Brew. Chem. 42 (1984) 54.
[9] L. De Cooman, E. Everaert, D. De Keukeleire, Phytochem. Anal. 9 (1998) 145.
[10] J. Freundorfer, Monatsschr. Brauwiss. 41 (1988) 312.
[11] M. Verzele, J. Inst. Brew. 92 (1986) 32.
[12] G.A. Howard, J. Inst. Brew. 76 (1970) 381.
[13] C.L. Arthur, J. Pawliszyn, Anal. Chem. 62 (1990) 2145.
[14] T. Górecki, in: J. Pawliszyn (Ed.), Applications of Solid Phase Microextraction, Royal Society of Chemistry, Cambridge, 1999, p. 93, Chapter 7.
[15] Z. Zang, J. Pawliszyn, Anal. Chem. 65 (1993) 1843.
[16] H. Prosen, L. Župančič-Kralj, Trends Anal. Chem. 18 (1999) 272.
[17] A.J. Matich, D.D. Rowan, N.H. Banks, Anal. Chem. 68 (1996) 4114.
[18] H. Kataoka, H.L. Lord, J. Pawliszyn, J. Chromatogr. A 880 (2000) 35.
[19] J.A. Field, G. Nickerson, D.D. James, C. Heider, J. Agric. Food Chem. 44 (1996) 1768.
[20] NIST/EPA/NIH Mass Spectral Library, Standard Reference Database 1A, National Institute of Standards and Technology, Gaithersburg, MD, 1995.
[21] R.P. Adams, Identification of Essential Oils By Ion Trap Mass Spectroscopy, Academic Press, 1989.
[22] A. Antonelli, C. Fabbri, Chromatographia 49 (1999) 125.
[23] J. Pawliszyn, in: J. Pawliszyn (Ed.), Applications of Solid Phase Microextraction, Royal Society of Chemistry, Cambridge, 1999, p. 3, Chapter 1.
[24] R.G. Buttery, R.E. Lundin, L. Ling, J. Agric. Food Chem. 15 (1967) 58.