Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1084 (2005) 2–6
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Comparison of three different solid-phase microextraction fibres for analysis of essential oils in yacon (*Smallanthus sonchifolius*) leaves

M. Adam*, M. Juklová, T. Bajer, A. Eisner, K. Ventura

Department of Analytical Chemistry, Faculty of Chemical Technology, University of Pardubice, nam.Cs. legii 565, 532 10 Pardubice, Czech Republic

Available online 17 June 2005

Abstract

A headspace solid-phase microextraction (HS-SPME) procedure based on three commercialised fibers (100 μm polydimethylsiloxane, 65 μm polydimethylsiloxane–divinylbenzene and 50/30 μm divinylbenzene–Carboxen–polydimethylsiloxane) is presented for the determination of a selected essential oils in dried leaves of yacon (*Smallanthus sonchifolius)*. The extraction performances of these compounds were compared using fibers with one, two and three coatings. The optimal experimental procedures for the adsorption and desorption of target compounds were determined. Significant parameters affecting sorption process such as sample weight, sorption and desorption time and temperature were optimised and discussed. Finally, the optimised procedures were applied successfully for the determination of these compounds in various yacon species. The relative concentration factors of three characteristic components of yacon were measured for relative evaluation of the fiber efficiency. Main essential oils were isolated from dried yacon leaves by appropriate solid-phase microextraction fiber and semi-quantitative analysis of the target volatiles was conducted by gas chromatography–flame ionisation detection (GC–FID) using a capillary column. Three compounds—β-pinene, caryophylene and γ-cadinene were found as the predominant essential oils. Its relative content was important for specification of yacon varieties. Solid-phase microextraction in combination with gas chromatography enabled a rapid and simple determination of relative content of essential oils in yacon.

Keywords: Yacon (*Smallanthus sonchifolius)*; Essential oils; Solid-phase microextraction

1. Introduction

Yacon (*Smallanthus sonchifolius*) is a tuber crop originally cultivated in South America in the Andean highlands. This plant constitutes part of the diet of Indian tribes living in the high mountain areas. It is food as well as acknowledged medicinal plant for the local population and has gradually received more attention due to its abundant content of fructooligosaccharides and phenolic compounds. Tubers are used as a source for the production of natural sweeteners and syrups suitable for persons suffering from digestive problems [1]. Yacon tubers contain a high concentration of oligofructans and the plant drew attention as a natural source for the supply of oligofructans [2]. Antidiabetic properties have been attributed to yacon leaves, which are dried and used in the preparation of tea. Antifungal activities of the leaves extracts have recently been reported. These bioligical activities are related to the essential oils content in each part of plant [3]. The tubers contain fructose, glucose, saccharose and inulin type oligofructans [2,4,5]. Aerial part should contain some antifungal and pesticidal compounds, as it is not necessary to use pesticides in the cultivation of Yacon. Both parts of this plant (leaves and tubers) represent a rich source of phenolic acids and other radical scavenging compounds. In Europe, the crop has been introduced for cultivation in Czech Republic [6].

Modern trends in analytical chemistry are towards the simplification and miniaturization of sample preparation, and the minimization of organic solvent used. However, liquid–liquid extraction (LLE) is procedure, which requires large amounts of toxic and expensive solvents that can be harmful to the operator and to the environment, is time consuming and tedious since it also requires pre-concentration of the extract

* Corresponding author. Tel.: +420 466037001; fax: +420 466037068.
E-mail address: martin.adam@upce.cz (M. Adam).

0021-9673/$ – see front matter © 2005 Published by Elsevier B.V.
doi:10.1016/j.chroma.2005.05.072

and it is at this stage that the potential for loss of analytes or contamination of samples is greatest.

As a modern alternative to traditional sample preparation technology has been introduced solid-phase microextraction (SPME). This technique is a extraction method developed by Pawliszyn and coworkers in 1989 [7–9]. It eliminates the use of organic (toxic) solvents, and substantially shortens the time of analysis when allowing one a convenient automation of the sample preparation step [10–14]. SPME can integrate sampling, extraction, concentration and sample introduction into a single uninterrupted process, resulting in high sample throughput. Its important features are simplicity, low cost, rapidity, selectivity and sensitivity when combined with appropriate detection modes [10,11,15]. One of the critical aspects on SPME optimisation is the selection of the appropriate fibre. SPME is used as a prior sample preparation stage, not only in gas chromatography, but in liquid chromatography [16] and capillary electrophoresis [17] as well.

This method has attracted widespread popularity for the analysis of volatile flavour components in various plants, food, beverage, natural product matrices, biological and environmental samples [18–21] and now it's widely accepted as a reliable technique. An et al. recently reported SPME–GC–MS for the analysis of fragrances from lavender flowers [22].

The present study was carried out to evaluate the performance of three different commercially available SPME fibers in the extraction of essential oils in yacon leaves using head-space SPME. The experimental parameters that affect the adsorption and desorption processes were evaluated. The extraction efficiencies were optimised by adjusting the following parameters: extraction time and temperature, amount of sample for extraction, temperature and time of desorption. Finally, the optimised SPME procedures were applied by employing GC with FID to determination of relative content of three most important essential oils in yacon leaves. Based on the relative content of these compounds it is possible to determine the yacon (*S. sonchifolius*) variety.

2. Experimental

2.1. Plant material and reagents

S. sonchifolius (yacon) plants, originally purchased from Ecuador, were obtained from the Institute of Medical Chemistry and Biochemistry (Palacky University of Olomouc, Czech Republic). Samples were harvested during the seasons from 1999 to 2002. The leaves were collected at harvest time, dried at room temperature and stored at 4 °C prior to use. These samples were entitled as follows: October 1999 and 2001, April 2001, August 2001 and Clone 5, 17, 18, 25, 28, 31, 64 and 85.

Standards of essential oils, myrcene and humulene, used for identification of target compounds were purchased from Sigma-Aldrich (Prague, Czech Republic) with purity >90%. The individual stock solutions were prepared in methanol at a concentration of 1000 mg l^{-1} and stored at 4 °C. Methanol of analytical grade was obtained from Merck (Darmstadt, Germany).

2.2. Instrumentation

All extracts obtained by SPME were analysed by GC–FID system HP-5890 (Hewlett-Packard, Avondale, PA, USA) equipped with capillary column Ultra No. 2 (25 m × 0.32 mm I.D., 0.52 μm film thickness of phenylmethylsilicone). Evaluations of chromatograms were performed with the use of integration software CSW (Data Apex Prague, Czech Republic). For identification of individual essential oils GC–MS system GC 17A coupled with MS detector QP 5050A (Shimadzu, Kyoto, Japan) were used. Extraction temperature was adjusted by thermostat Julabo EC-5 (Julabo Labortechnik, Seelbach, Germany).

2.3. SPME fibres and extraction conditions

Three different coated SPME fibers, 100 μm polydimethylsiloxane (PDMS), 65 μm polydimethylsiloxane–divinylbenzene (PDMS–DVB) and 50/30 μm divinylbenzene–Carboxen–polydimethylsiloxane StableFlex (DVB–CAR–PDMS) were used. The commercially available SPME device for manual sampling and all fibers were purchased from Supelco (Bellefonte, PA, USA). Before use, SPME fibers were reconditioned by heating in hot injection port of a gas chromatograph at 220 °C for 30 min in order to remove contaminants.

All extractions were performed in 8 ml dark glass SPME vials sealed with a PTFE faced septum caps (Supelco, Bellefonte, PA, USA). Prior to extraction, analysed samples were pulverised in the friction dish to the small sized powder. Appropriate SPME fibre was exposed to the headspace above 0.5 g of dried sample contained in a 8 ml SPME vial. Preliminary experiments using fibre exposition times between 5 and 70 min and temperatures between 30 and 90 °C were used to determine the optimal extraction conditions.

2.4. Chromatographic analysis

After extraction, the SPME fiber was removed from the sample vial and immediately inserted into the injection port of the gas chromatograph using an SPME-liner where the thermal desorption occurs at 220 °C for 5 min. Separation conditions were as follows: initial column temperature 60 °C, increased to 150 °C at 5 °C min^{-1} and, finally, increased to 280 °C at 30 °C min^{-1} (hold 2 min). The injector and detector temperatures were maintained at 220 and 190 °C, respectively. The nitrogen (purity 5.0, Linde Technoplyn, Prague, Czech Republic) was used as carrier gas with 40 kPa column head pressure at a flow-rate of 1.7 ml min^{-1} (split ratio of 1: 10). Hydrogen and air (both Linde Technoplyn, Prague,

Czech Republic) at 30 and 300 ml min^{-1}, respectively, were used in the FID.

3. Results and discussion

The aim of this investigation was to gain qualitative and semi-quantitative essential oils profiles for direct comparison of various yacon (*S. sonchifolius*) varieties. The relative content of at least three of main essential oils is impotrant for characterisation of various yacon varieties. As a predominant essential oils of yacon leaves β-pinene, caryophylene and γ-cadinene were found. Chemical structures of mentioned compounds are shown on the Fig. 1.

3.1. Optimisation of SPME conditions

To develop a SPME procedure for the determination of essential oils, optimisation of several variables related to the extraction and desorption steps is required (SPME fiber selection, sample weight, extraction time and temperature as well as the time and temperature of desorption) in order to achieve maximum efficiency of extraction of the compounds studied and to resolve the selectivity of the different coatings versus other components present in the matrix.

3.1.1. Selection of SPME coating

SPME is a process dependent on the equilibrium process involving partitioning of the analytes from the sample into the stationary phase and the extraction time depends on the type of stationary phase used. Three SPME fiber coatings were evaluated to the most suitable for the HS-SPME–GC–FID determination of essential oils.

With the aim of comparing the extraction efficiencies of the three different commercially available SPME fibers (100 μm PDMS, 65 μm PDMS–DVB and 50/30 μm DVB–CAR–PDMS) were studied. Fig. 2 displays the comparison of chromatograms obtained for each of the fibres tested. All extractions were performed at 80 °C for the time interval 40 min.

The best results being obtained for most of the target compounds with the fiber DVB–CAR–PDMS, which can be explained not only by the nature of the fiber, but by slightly larger capacity for the analytes. Using the DVB–CAR–PDMS fibre allows the simultaneous extraction of target compounds, with a single SPME procedure and analysed in a single chromatographic run with an instrumental

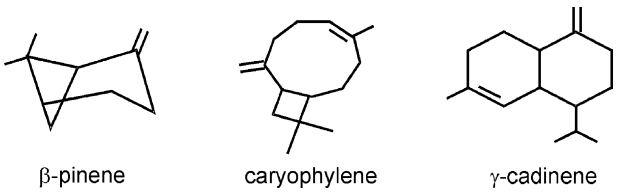

Fig. 1. Chemical structure of target essential oils.

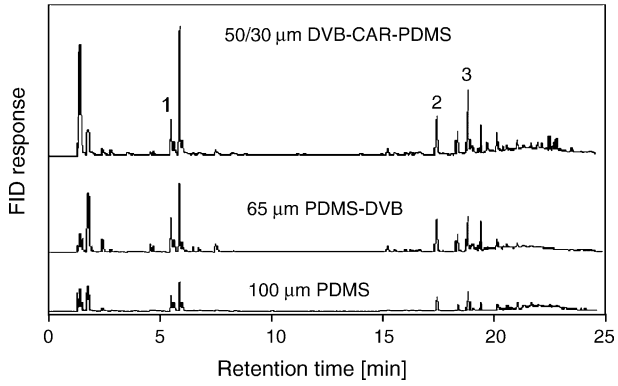

Fig. 2. Comparison of chromatograms obtained for tested SPME fibres. (1) β-pinene, (2) caryophylene, (3) γ-cadinene.

configuration of GC–FID. The fibre maintains its performance well for >100 extractions with between-day precision below 10%.

3.1.2. Effect of temperature

The effect of the extraction temperature on the HS-SPME efficiency was tested. In this investigation, temperatures varied every 10 °C from 30 to 90 °C with the DVB–CAR–PDMS fiber. At this evaluation stage, the extraction time was set at 40 min in order to obtain equilibrium. Dependencies of GC–FID peak areas on extraction temperature for three components such as β-pinene, caryophylene and γ-cadinene are shown in Fig. 3.

It was observed that β-pinene extraction decreased with temperature. Caryophylene and γ-cadinene using the selected fiber at 80 °C showed relatively the highest peak areas. In subsequent studies, this temperature was selected as the extraction temperature.

3.1.3. Sorption time profile

HS-SPME is considered complete when the analyte concentration has reached equilibrium between the sample matrix and the fiber coating. Determination of adsorption equilibrium times for β-pinene, caryophylene and γ-cadinene as the test references was carried out using the DVB–CAR–PDMS. In this investigation, adsorption times were varied 5–70 min at 80 °C. The extraction time profile

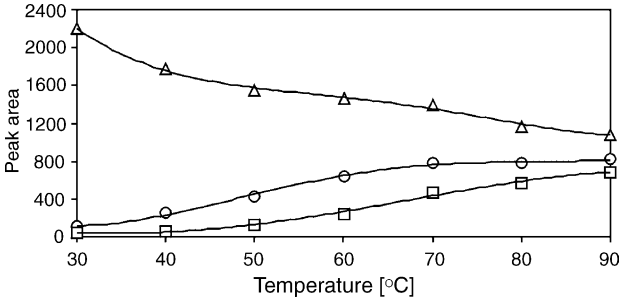

Fig. 3. Effect of extraction temperature on the HS-SPME-GC of target essential oils of yacon. (△) β-pinene, (◯) caryophylene, (□) γ-cadinene.

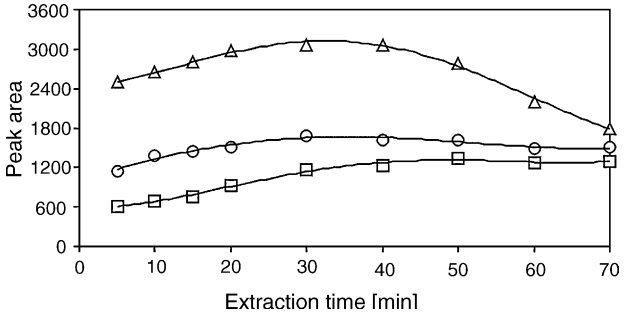

Fig. 4. Effect of extraction time on the HS-SPME–GC of target essential oils of yacon. (△) β-pinene, (◯) caryophylene, (□) γ-cadinene.

was obtained by plotting the FID response versus the extraction times evaluated (Fig. 4).

In the case of selected SPME fiber the optimum extraction time was 40 min and no significant improvement in the extraction was obtained at longer times where decreasing amount of β-pinene is due to competitive sorption of the others compounds contained in yacon. In subsequent studies, this time was selected as the extraction time.

3.1.4. Desorption conditions

The following step in the optimisation process was to select the optimum desorption conditions, which were determined by testing different temperatures and times, considering the optimum values to be those obtained when all analytes were desorbed from the fiber coating with minimal carryover effect to the following analysis. For the DVB–CAR–PDMS fiber, desorption temperatures ranged between 200 and 250 °C. The time of desorption was also optimised, varying between 1 and 15 min. Stabilization of the chromatograms was observed and reproducible peak areas were obtained for the references mixture using desorption time of 5 min at 220 °C. These values of temperature and desorption time were selected for subsequent studies.

3.1.5. Total content of essential oils

The total content of essential oils in yacon leaves was determined by steam distilation and was found at the level of 0.1% (m/m) with RSD = 2.5%. These experiments were performed using approx. 40 g of dried sample and 600 ml of water with duration of 4 h.

3.1.6. Validation of evaluated method

For the method described above, the limits of detection were found LOD = 0.1 mg of each essential oil per 1 g of plant material. The limits of quantification (LOQ) of each target compound were determined at the level 0.3 mg per 1 g of plant material. The limits of detection were calculated as $LOD = 3 \times N$, where N is the baseline noise at the appropriate retention time with areas equal to the mean value of five blanks. The limits of quantification were calculated as $LOQ = 10 \times N$. These limits seems to be higher in the contrary to minimise of detection limits of analytical procedure.

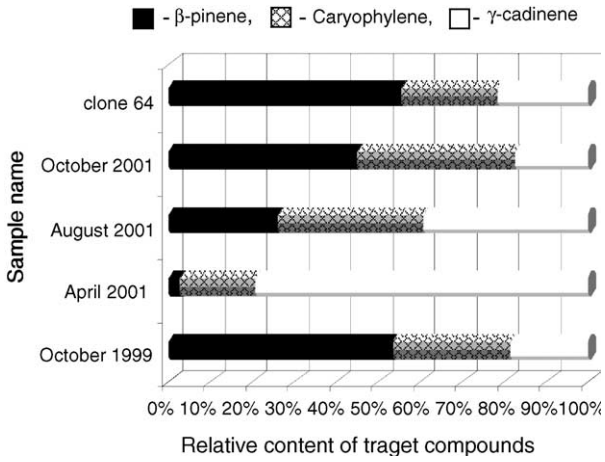

Fig. 5. Relative content of target essential oils in various yacon samples–mixed samples. The vertical axis expresses the name of each analysed sample.

But the aim of this study was diferentiation of various yacon species based on essential oils profile. Our limits of detection and quantitation are low enough for this study.

3.2. Application to real samples

The optimised SPME method for the DVB–CAR–PDMS fiber was applied successfully to the analysis of various yacon varieties with the aim of finding out the variances in relative contents of target essential oils. For application of dried yacon leaves, 80 °C extraction temperature and 40 min extraction time were finally used. All samples were analysed five times. The relative standard deviation RSD = 5% was determined. Confirmation of the peaks was realised using a MS detector and comparing with the standards of myrcene and humulene. For each target compounds the relative content was only determined according to the aim of this study. All results were calculated like the amount of each compound related to the

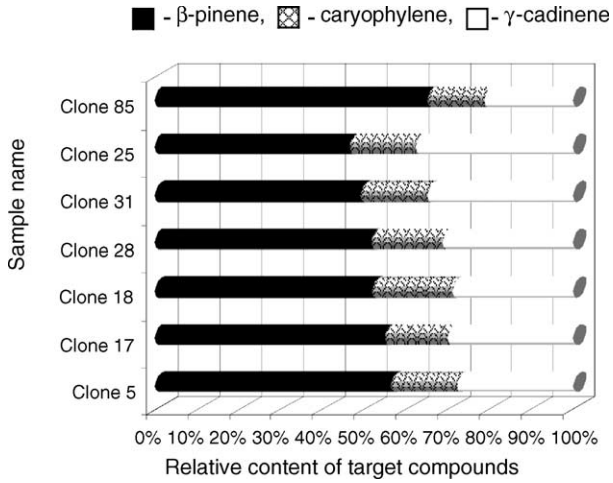

Fig. 6. Relative content of target essential oils in various yacon samples–pure samples. The vertical axis expresses the name of each analysed sample.

sum of amounts of analysed essential oils. Results obtained by these experiments are shown on Figs. 5 and 6.

According to very similar relative content of target essential oils it is possible to decide that these samples (Clone 5 conformable to Clone 17 and Clone 18 conformable to Clone 28) are the same or very similar varieties of yacon.

4. Conclusions

The SPME method used for the extraction of yacon volatiles ensured qualitative differentiation of the yacon varieties. SPME combined with GC–FID was found to be suitable for the determination of essential oils in dried yacon leaves. This technique is simple, economical, do not require any preliminary sample preparation step and reduce the volume of (toxic) solvents used.

Usually the process of fibre type selection is made based on the nature and thickness of the polymeric coating. Internal sublayers that exist in PDMS–DVB fibres can have an important role in the selectivity of the fibre towards small differences in the polarity of analytes. The process of SPME fibre selection for a particular application cannot be entirely dependent on product information but based on a deep knowledge of inherent properties of the fibre.

In our target compounds 30/50 μm DVB–CAR–PDMS fibre gives the best combination of sensitivity fulfilling the requirements of the method for yacon analysis. The differences in selectivity provided by the different coatings can be used not only for quantification purposes, but also for identification of these compounds in complex samples.

The optimal extraction conditions for selected SPME fiber were: sample weight 0.5 g, volume of the sampling vial 8 ml, extraction time 40 min, extraction temperature 80 °C, desorption temperature 220 °C, desorption time 5 min. The optimised SPME procedure was successfully applied to the analysis of three most important essential oils (β-pinene, caryophylene and γ-cadinene) in dried yacon leaves. Analysis of these three essential oils seemed to be sufficient for the differentiation of various yacon varieties.

Acknowledgements

Authors thank for financial supports from the Ministry of Education, Youth and Sports of the Czech Republic (Project MSM 0021627502) and from the Czech Science Foundation (Project No. 203/05/2106 and No. 203/02/D002).

References

[1] A. Grau, J. Rea, H. Robinson, in: M. Hermann, J. Heller (Eds.), Andean Roots and Tubers, Ahipa, Arracha, Maca and Yacon, IPGRI, Rome, 1997, p. 199.
[2] T. Ohyama, O. Ito, S. Yasuyosi, T. Ikarashi, K. Minamisawa, M. Kubota, T. Tsukihashi, T. Asami, Soil Sci. Plant Nutr. 36 (1990) 167.
[3] A. Inoue, S. Tamogami, H. Kato, Y. Nakazato, M. Akiyama, O. Kodama, T. Akatsuka, Y. Hashidoko, Phytochemistry 39 (1995) 845.
[4] T. Asami, M. Kubota, K. Minamisawa, T. Tsukihashi, Jpn. J. Soil Sci. Plant Nutr. 60 (1989) 122.
[5] K. Goto, K. Fukai, J. Hikida, F. nanjo, Y. Hara, Biosci. Biotech. Biochem. 59 (1995) 2346.
[6] B. Simonovska, I. Vovk, S. Andrenšek, K. Valentová, J. Ulrichová, J. Chromatogr. A 1016 (2003) 89.
[7] C.L. Arthur, J. Pawliszyn, Anal. Chem. 62 (1990) 2145.
[8] Z. Zhang, J. Pawliszyn, Anal. Chem. 65 (1993) 1843.
[9] J. Pawliszyn, Trends Anal. Chem. 14 (1995) 113.
[10] J. Pawliszyn, Solid-Phase Microextraction: Theory and Practice, Wiley-VCH, New York, 1997.
[11] J. Dugay, C. Miege, M.-C. Hennion, J. Chromatogr. A 795 (1998) 27.
[12] M. de Fatima Alpendurada, J. Chromatogr. A 889 (2000) 3.
[13] H. Lord, J. Pawliszyn, J. Chromatogr. A 885 (2000) 153.
[14] D. Barcelo, M.-C. Hennion, Trace Determination of Pesticides and their Degradation Products in Water, Elsevier, Amsterdam, 1997.
[15] Z. Zhang, M. Yang, J. Pawliszyn, Anal. Chem. 66 (1994) 844A.
[16] S.H. Salleh, Y. Saito, Y. Kiso, K. Jinno, Anal. Chim. Acta 433 (2001) 207.
[17] K. Jinno, Y. Han, H. Sawada, M. Taniguchi, Chromatographia 46 (1997) 309.
[18] A. Jayatilaka, S.K. Poole, C.F. Poole, T.M.P. Chinchila, Anal. Chim. Acta 302 (1995) 147.
[19] R.P. Belardi, J. Pawliszyn, Water Pollut. Res. J. Can. 24 (1989) 179.
[20] J. Cai, B. Liu, Q. Su, J. Chromatogr. A 930 (2001) 1.
[21] C.A. Zini, H. Lprd, E. Christensen, T.F. Assis, E.B. Caramao, J. Pawliszyn, J. Chromatogr. Sci. 40 (2002) 140.
[22] M. An, T. Haig, P. Hatfield, J. Chromatogr. A 917 (2001) 245.