THESIS

Cultivation of Herbs and Medicinal Plants in Norway – Essential Oil Production and Quality Control

by

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Submitted for the degree of Dr. philos.

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1. PREFACE AND ACKNOWLEDGEMENTS

This thesis has been written and researched at The Plant Biocentre, Department of Biology, Norwegian University of Science and Technology (NTNU), during the period of July 1997 to December 2002, however had its real starting point with the establishment of the project and later foundation "Norwegian Herb Production (Norsk Urteproduksjon NUP)" in 1994.

This work is based on five scientific articles (Papers B-F) published in international journals during the period between 1999 and 2002, and these establish the backbone of the thesis. The introductory chapter is represented by a review article on aromatic plants and essential oils (Paper A), which is preaccepted and will be published within the next three months. The contents list of Paper A remains unchanged and thus, the contents of the thesis are listed briefly, referring only to the presented papers and not to their chapters and sections.

The entire topic of Paper A has been elaborated in a comprehensive and in-depth basis in order to describe biological functions, applications and different ways of isolating and analysing essential oils. The author has introduced *solid-phase microextraction* (SPME) coupled with gas chromatography-mass spectrometry (GC-MS) in the routine and research work on aromatic and medicinal plants at The Plant Biocentre (see Papers B, C, E and F). Thus, a great deal of focus has been on the applicability of SPME for the analysis of terpene volatiles from aromatic plants, which is highlighted by numerous examples from the author's lab. In addition, results from the NUP-project in the period from 1994-1996 have been referred to in Paper A to outline the project's major results, giving a better understanding of the cultivation of herbs and medicinal plants in Norway and the production of essential oils.

When I moved to Trondheim in September 1993 to start my botanical studies at the Department of Botany located at that time on Rosenborg, I met Prof. Tor-Henning Iversen and his research group working with aromatic and medicinal plants. Although I was not directly involved in the initial part of the project work of the NUP-project, my cand.scient. thesis on the production and quality of rasperry leaves dealt with actual questions and topics related to this project. Throughout my study and later on at The Plant Biocentre (1995), while working as a project co-worker for NUP and other herb and medicinal plant-related projects, Prof. Iversen has been of invaluable help. In the fulfilment of this thesis, he has been a source of inspiration and critical discussion, and his enthusiasm and ideas deserve my greatest appreciation.

I would like to thank all my colleagues at The Plant Biocentre who have directly or indirectly been involved in the elaboration of this work and who throughout the past five years have created a pleasant and inspiring environment both at the centre and socially. I especially want to thank Guri Fyhn Hanssen and her "green hands" for assisting in the cultivation of aromatic plants in the greenhouse and the trial fields at Dragvoll, and Grete Rakvåg for her assistance of my research work in the lab with regard to sample preparation and distillation.

I would also like to thank the foundation ALLFORSK and director Marit Svendsen for financially supporting the completion of my thesis through a one-month grant in April 2002, as well as all the other staff members at ALLFORSK for their complaisant and effective project coordination.

The helpfulness of Janet Iversen for reading and correcting my English manuscripts and reports is much appreciated.

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I am grateful to my family: to Wenche whom I married on August 3rd this year, her love, inspiration, patience and hours of rest together, which made the completion of this thesis possible, and to Gabriel, Maria, Peder and Adrian for showing me alternative and entertaining ways of using my laptop during my stressful homework.

I would like to thank my family in Germany, my mother and my sister, and my in-laws for their interest in my work, and their frankness and understanding for my being busy with scientific work during the last year. Finally, I would like to thank all my friends in Trondheim and afar, and all my “guinea pigs” that have shown curiosity for and confidence in phytomedicine and the healing properties of essential oils. After all, they work!

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2. GENERAL SUMMARY

Essential oils (EO) are plant secondary metabolites that are known for their fragrance and food flavour properties. They consist of a complex mixture of mono- and sesquiterpenes, phenyl propanoids and oxygenated compounds. EOs can be present in different plant organs and materials, and their storage is related to specialised secretory structures. The yield of EOs from plant raw materials by distillation or pressing may on average vary from 0.1 – 1%, thus restricting the major EO production to the plant group of aromatic plants. Due to their function as signalling compounds between different types of organisms and diverse biological systems, their general antimicrobial and antioxidative effects and medicinal activity, EOs offer a promising potential for future applications within the fields of agriculture, medicine, pharmaceutical industry and biotechnology.

Changed consumer demands and raised interest in natural product compounds, especially essential oils, have formed the basis for initiating the research project "Norwegian Herb Production (Norsk Urteproduksjon NUP)" to encourage the cultivation, processing, marketing and distribution of aromatic and medicinal plants. The production, composition and quality characteristics of EOs (yield and terpene composition) from chamomile, lemon balm, oregano, peppermint, sachalinmint, thyme and yarrow have been investigated in the project period between 1994-1998.

Much focus has been put on the application of *solid-phase microextraction* (SPME) coupled with gas chromatography-mass spectrometry (GC-MS) for the analysis of EO volatiles from various aromatic and medicinal plants. SPME is a fast, solvent-free and non-destructive sample preparation technique where the analytes are extracted from fluid or solid matrices by *headspace* (HS) or *direct immersion* sampling (DI). Apart from EO isolation by common distillation, the applicability and sensitivity of the SPME fibre has made it feasible to carry out qualitative and semi-quantitative HS analyses of aromatic plants with regard to changes of EO metabolism during ontogenesis and plant development.

Based on NUP-results from field trials in the period between 1995-1996, the mint species peppermint (*Mentha* × *piperita* L.) and sachalinmint (*Mentha sachalinensis* (Briq.) Kudô) have been studied in detail (Papers B, D and E). Comparative analyses by applying distillation sampling and SPME have been carried out in order to study the advantages and disadvantages of both techniques (Papers B and E). It could be shown, that SPME offers a fast and reliable method for detecting quality-impact compounds from the *p*-menthane group (menthol, menthone, neomenthol, isomenthone and menthyl acetate). A distinct increase in the menthol/menthone ratio in the basipetal direction could be detected for peppermint and sachalinmint by applying SPME, thus revealing within-plant quality differences according to pharmacopeial requirements. Taking the increase of EO production from the vegetative to the generative growth stage into account, the harvest of mint plants in bloom will result in better EO yield and quality with regard to higher amounts of menthol.

When applying HS-SPME on complex EO volatile matrices such as known for yarrow (*Achillea millefolium* L.; Paper C), one might deal with fibre-partitioning effects of the different mono- and sesquiterpenes due to their physical and chemical properties. Despite these disadvantages, HS-SPME appears to be a sensitive extraction method for the screening of EO volatiles from complex sample matrices. Comparative analyses of volatiles from rose root rhizomes (*Rhodiola rosea* L.) have been carried out in order to characterize the rose-like

odour compounds (Paper F). A total of 75 and 59 compounds have been identified by distillation sampling and HS-SPME, respectively, thus underscoring the excellent extraction properties and applicability of the SPME fibre.

Paper A gives a brief overview of EO biosynthesis and chemical structures, plant sources and methods of EO production. Before leading over to the main topic of HS-SPME applications by referring to numerous examples from the research work at The Plant Biocenter in the past 5 years, an introduction of solid-phase microextraction with regard to devices, procedures and extraction parameters is given.

The advantages and disadvantages of distillation vs. SPME are outlined on the background of comparative analyses of peppermint, chamomile, basil and dill. Furthermore, the utilization of HS-SPME for quantitative studies with regard to extraction time and analyte concentration is being highlighted. Examples for the screening of chemotypes (hops – *Humulus lupulus* L.) and cultivars (dill – *Anethum graveolens* L.) and ontogenetic studies are given (*Mentha* species; arnica – *Arnica montana* L.). Finally, the applicability of HS-SPME for the quality assessment of processed herbs (sweet basil – *Ocimum basilicum* L.) and phytomedicinal preparations (red coneflower – *Echinacea purpurea* L.) is being discussed.

The advantages of HS-SPME over classical distillation and headspace applications are impressive due to drastically reduced analysis time and will introduce new frontiers in plant volatile research with regard to secondary metabolism, plant-insect interactions and *in vivo* studies. The user-friendliness of operating SPME will initiate the development of future applications and equipment for the monitoring of volatiles for plant biological and environmental studies, extraction automation, on-site sampling and on-fibre storage of analytes.

ESSENTIAL OIL DRUGS – TERPENE COMPOSITION OF AROMATIC HERBS[1]

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CONTENTS

Abbreviations: ASE: Accelerated Solvent Extraction; CAR: Carboxen; CW: Carbowax; DHS: Dynamic Headspace; DI: Direct Immersion; DTD: Direct Thermal Desorption; DVB: Divinylbenzene; EO: Essential Oil; FID: Flame Ionization Detector; GC: Gas Chromatography; HD: Hydrodistillation; HS: Headspace; HPLC: High-Performance Liquid Chromatography; HSSE: Headspace Sorptive Extraction; LC: Liquid Chromatography; MAE: Microwave-Assisted Extraction; MAPs: Medicinal and Aromatic Plants; MS: Mass Spectrometry; NPD: Nitrogen Phosphorus Detector; PA: Polyacrylate; PCR: Polymerase Chain Reaction; PDMS: Polydimethylsiloxane; SD: Steam Distillation; SDE: Solvent-Distillation Extraction; SE: Solvent Extraction: SFE: Supercritical Fluid Extraction; SHS: Static Headspace; SPE: Solid-Phase Extraction; SPME: Solid-Phase Microextraction; VOC: Volatile Organic Compounds

[1] This article will establish the introductory section of the authors Dr.philos-thesis entitled «Cultivation of Herbs and Medicinal Plants in Norway - Essential Oil Production and Quality Control». Thus, the entire topic will be elaborated on a comprehensive and in-depth basis.

1. INTRODUCTION

"Essential Oils are the Winds of Gods and we Earthly Souls are the Chosen Ones to be tempted by their Enticing Breath" — Nothing is more beguiling to our senses and touches us more intimately than the fragrance from flowers and aromatic essences. But what mesmerizes us into that state of pleasant sensation and dreaming and why do we perceive other kinds of scents as unpleasant, let alone disgusting? Question about the nature of things and their material composition are surely as old as the attempts to give the answers meaning through religious faith and metaphysical explanations. The search for knowledge and intellectual curiosity are a basic human traits and, naturally, answers are not long coming. However, the kind of results we get from our investigations, not only depend on the questions proposed, but are equally determined by the means and tools we make use of to achieve satisfactory results. Seen from a scientific point of view, researchers have at their disposal a vast tool box of analytical instruments and devices for studying essential oils (EO).

Raw materials for essential oil production often come from temperate or Mediterranean climates, although terpene structures appear throughout the plant kingdom all over the world and can even be found in species growing under unfavourable conditions in deserts or arctic climates. The economic importance of aromatic plants can be traced back to the ancient world when essences and fragrances were sold as valuable goods. Archaeological discoveries revealed evidence that the basic knowledge for EO production by distillation has been known for over 4000 years. The essences from the Orient, Arabia and South-Asia were handled as treasures and sold on the European markets in ancient Greece and in Old Rome. Not only were the fragrance and food flavour properties of aromatic plants interesting to the alchemists of the Renaissance who began studying indigenous plants of Central Europe, the medicinal attributes of EOs were especially attractive. The invention of the printing press in the 15th century resulted in the knowledge and wisdom that had been recorded in various herbal works being printed between 1450 and 1650.

The later Industrial Revolution went hand-in-hand with the scientific revolution in replacing alchemy with modern chemistry based on logical deductions and analytical methods. In the beginning of the 20th century, herbal and aromatic medicine lost much of its importance based on the discoveries of an up-coming, inventive pharmaceutical industry, which was introducing synthetic derivates of natural bioactive compounds. The implication of EOs for modern phytopharmacy was revived by laying the foundations for aromatherapeutical applications in France and England in the 1920s. Being a source for medicinal applications, EO quality requirements have been manifestated themselves in guidelines and monographs of phamacopeias in several countries and communities (e.g.

AFNOR, 1996; Council of Europe, 2002; Martindale and Sweetman, 2002). Changed consumer demands, raised public interest, health consciousness and, last but not least, the capability of EOs in biotechnology has blown new wind under the wings of modern phytomedicine.

These trends have also been recognized in peripheral areas of Europe such as Scandinavia, through the encouragement of cultivation, processing, marketing and distribution of aromatic herbs and medicinal plants. In Norway, the national project, "Norwegian Herb Production (NUP)", was carried out during the period from 1994-1998. The project was supported by the Norwegian Research Council (NFR) and national development funds, focussing on precisely these tasks to meet potential market opportunities. Production, composition and quality characteristics of EOs from chamomile, lemon balm, oregano, peppermint, sachalinmint, thyme and yarrow have been investigated (Iversen, 1994; Iversen et al. 1994a and 1994b; Iversen and Steen, 1996 and 1997; Iversen and Rohloff, 1997) and cultivation guidelines stressing geographic and climatic needs have been devised (Dragland and Galambosi, 1996; Iversen and Rohloff, 1998). The fundamentals of routine isolation, separation and analysis techniques for EOs and aroma volatiles in general were established at The Plant Biocentre and constituted the basis for a scientific network between university, research institutions and private companies. Aromatic herbs such as *Mentha* × *piperita* L. (Rohloff, 1999; Rohloff et al., 2000b), *Mentha sachalinensis* (Briq.) Kudô (Rohloff et al., 2000b; Rohloff, 2002a), *Achillea millefolium* L. (Rohloff et al., 2000a) and *Rhodiola rosea* L. (Rohloff, 2002b; Rohloff et al., 2002c) have been studied in detail by applying solid-phase microextraction (SPME) and gas chromatography-mass spectrometry (GC-MS) as the favoured analytical tools-of-choice.

The following gives an introduction into the fascinating world of essential oils (Chapter 2). An overview of developments and reports on recent essential oil research is given with focus on solid-phase microextraction (SPME) by outlining devices, procedures, extraction parameters and applicability for EO volatile detection within plant research and food quality control (Chapter 3). This review emphasises the applications of SPME by presenting many hitherto unpublished results from studies on aromatic plants carried out in the author's laboratory at the Plant Biocentre in Trondheim, Norway, over the past 5 years (Chapter 4) before proceeding to future prospects and trends within EO production, analysis and applications (Chapter 5).

2. Scent and Signal ✧ THE WORLD OF ESSENTIAL OILS

2.1 Structures and Biosynthesis

Plant primary products refer to the chemical groups of carbohydrates, proteins, nucleic acids, fats and lipids. Their functions are related to structure, physiology and genetics, which imply their crucial role in plant development. In contrast, secondary metabolites normally occur as minor compounds in low concentrations. They are involved in signalling mechanisms and plant defence and show quite characteristic patterns in their relationship to plant family or genus and thus, underscore their importance for chemotaxonomical and systematic determinations. Although many of these metabolites show structural similarities to primary products, one can divide secondary metabolites into the main chemical groups: *terpenoids*, *alkaloids*, *phenolics*, *rare amino acids*, *plant amines* and *glycosides*.

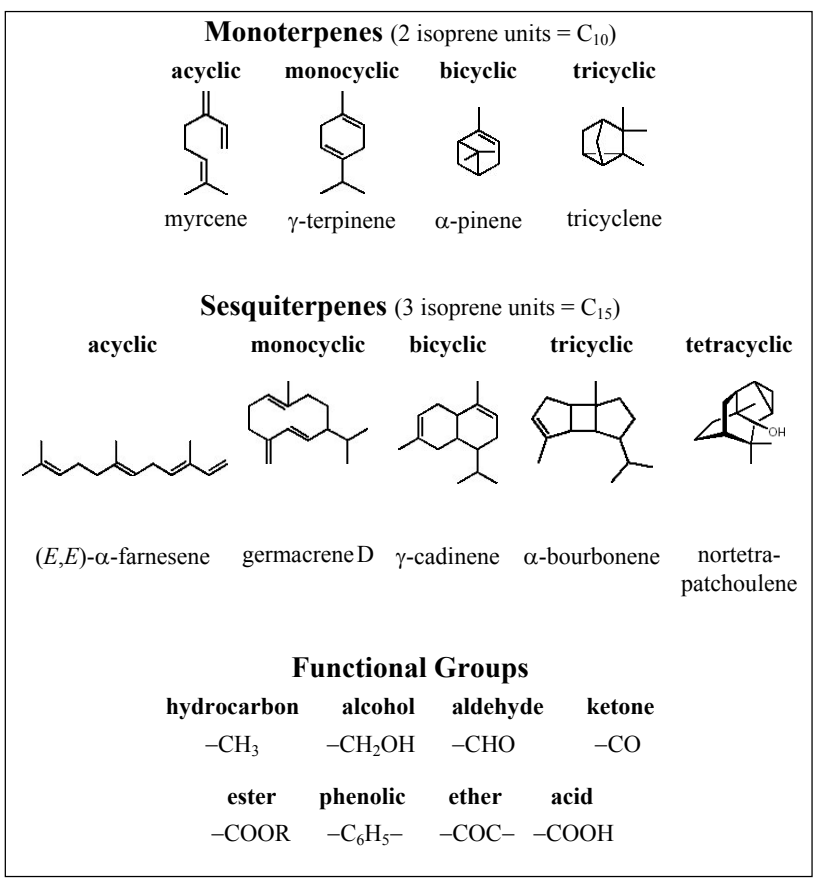
The chemical structure of the *terpenoids* is based on up to five C_5-*isoprene units* (CH_2=C(CH_3)-CH=CH_2), which lead to higher molecular structures of the *isoprenoids* through elongation and cyclization such as gibberellins, steroids, carotenoids and rubber, etc. The so-called biogenetic isoprene rule describes the basic carbon skeleton of terpenic and isoprenic structures by dividing them into the following classes:

monoterpenes	2 units	C_{10}
sesquiterpenes	3 units	C_{15}
diterpenes	4 units	C_{20}
sesterpenes	5 units	C_{25}
triterpenes	6 units	C_{30}
tetraterpenes	8 units	C_{40}
polyterpenes	> 8 units	

Newer findings provide evidence for the existence of compartimentation and enzymatic independence of two crucial biosynthesis pathways of terpenoids – the classical MAD route (*mevalonate-dependent*; Waterman, 1993; Schmidt, 1998; Kribii et al., 1999; Loza-Tavera, 1999) and the commonly accepted MAI route (*mevalonate-independent*; Rohmer et al., 1996; Keskitalo, 1999; Kreuzwieser et al., 1999; Sangwan et al., 2001; Dewick, 2002), also termed as the *deoxy-xylulose phosphate* (DOXP) pathway. In their multidisciplinary book about natural terpenoids, Harrewijn and co-authors (2001) give a detailed overview of possible ways for the evolutionary-originated development of biosynthetic pathways, discussing the alternatives for the production of the central terpenoid precursors *isopentenyl diphosphate* (IPP) and *dimethylallyl diphosphate* (DMAPP). Studies with the isotopic-labelled precursors DOXP and mevalonic acid

have been carried out (Piel et al., 1998) to describe the different pathways for monoterpenes (Lichtenthaler, 1999; Dewick, 2002). Monoterpenes, diterpenes and even more complex structures such as carotenoids are produced in plastides via *geranyl diphosphate* (GPP), whereas sesquiterpenes, triterpenes and sterols derive from cytosolic synthesis via *farnesyl diphosphate* (FPP). Despite their site-specific synthesis and complexity, monoterpenes and sesquiterpenes show structural similarities and form the main pool of compounds found in essential oils of aromatic herbs. Aliphatic hydrocarbons such as acids, aldehydes and acids and the phtaloids normally occur in lesser amounts. According to cyclization structure and functional groups, monoterpenes and sesquiterpenes can be divided into the following classes (Figure 2-1):

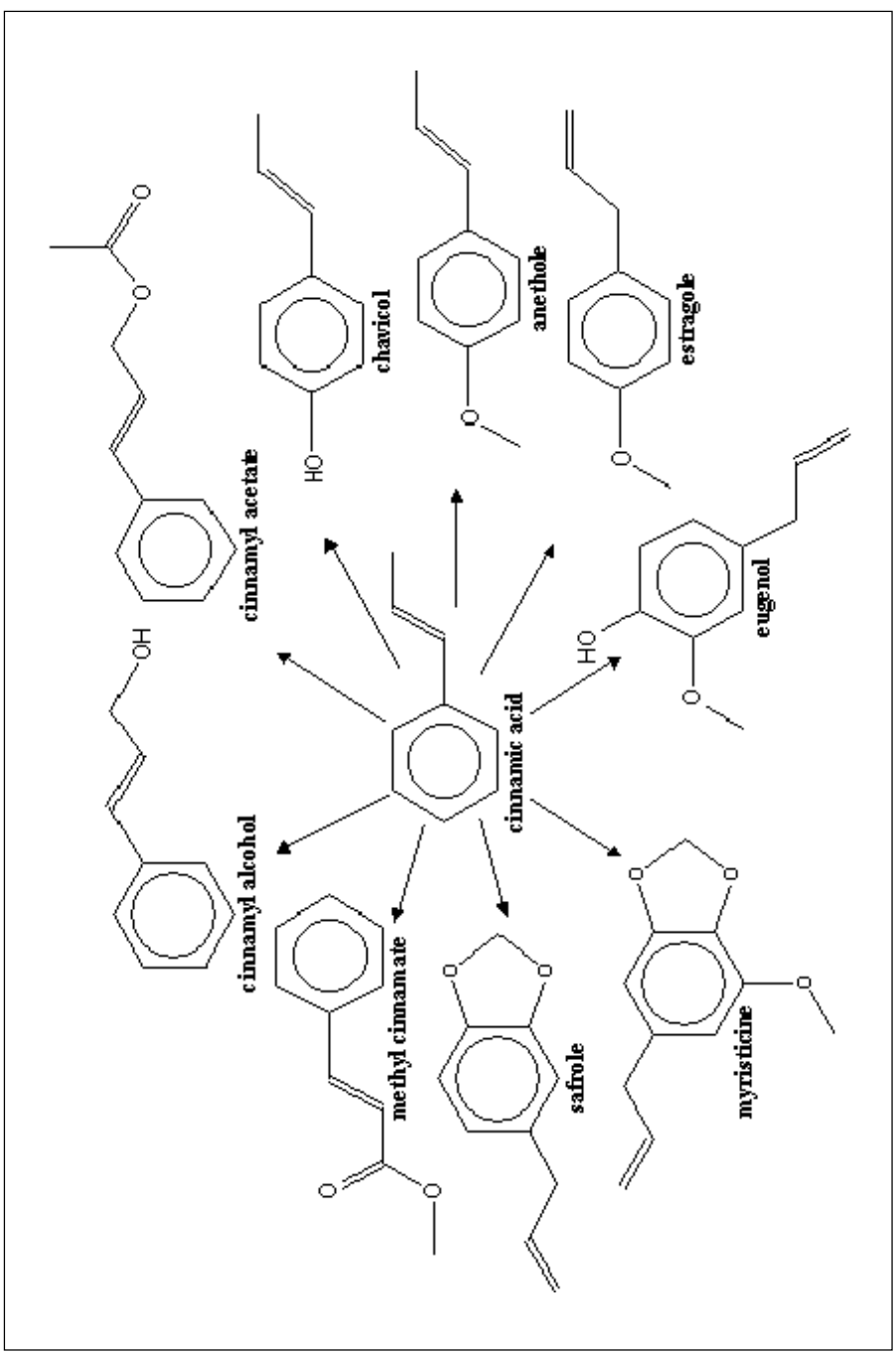
Figure 2-1: Classification of monoterpenes and sesquiterpenes.



High concentrations of phenylpropanoids or more strictly, phenylpropenes, can often be found in tropical spices. These compounds derive from the shikimic acid pathway and are based on the intermediate (*E*)-*cinnamic acid*, which leads to differentiated structures through side chain modification and functional groups, isomeric variation and different levels

of oxidation. Significant phenylpropenes are cinnamic acid esters, the isomers anethole and estragole, eugenol, myristicine, elimicine, safrole and apiole. Important phenylpropene structures due to flavour properties from commercially valuable herbs and spices are presented in Figure 2-2.

Figure 2-2: Important phenylpropenes of EOs found in herbs and spices.



2.2 Plant Sources and Production

Whilst the chemical structures of terpenoids can be found throughout the plant *and* animal kingdom, the occurrence of essential oils (EO) is restricted to over 2000 plant species from about 60 different families and only about 100 species are the basis for the economically important production of essential oils in the world (Lawless, 1996). Among the aromatic plants, spices, for example nutmeg, maze, cardamom and clove, mostly derived from tropical plants and used for the seasoning of foods, are especially well known for quite high essential oil concentrations of up to 20 % (Richards, 1991). One has to make a distinction between oils and oleoresins, which are a mixture of fats, waxes and EO, but often represent a higher marketable value of the plant extractives. The ability to accumulate EOs is quite high in both *Gymnosperms* and *Angiosperms*, although the most commercially important EO plant sources are related to the latter. Except for the conifers and junipers, most of the aromatic plants and EO commodities in terms of world trade are related to the families of *Labiatae*, *Umbelliferae* and *Compositae* (Hay and Svoboda, 1993).

Table 2-1: Examples of multi-purpose plants used for flavouring and EO production.

Botanical name	**English name**	**EO plant organs**	**Others**
Abies and *Picea* sp.	fir and spruce	needles and branches	oleoresins from wood (turpentine)
Angelica archangelica L.	angelica	roots, leaves and seeds	nutrient (leaves)
Arnica sp.	arnica	roots and flowers	-
Cinnamon camphora L.	camphor tree	wood and leaves	-
Cinnamon sp.	cinnamon	bark, stems and leaves	-
Cupressus sp.	cypress species	needles, branches and cones	-
Foeniculum vulgare Mill.	(sweet) fennel	seeds	nutrient (stem)
Juniperus sp.	juniper species	needles and berries	EOs and oleoresins from wood
Laurus nobilis L.	sweet bay (laurel)	leaves, twigs and berries	-
Levisticum officinale Koch.	lovage	roots, leaves and stems	nutrient (leaves)
Myristica fragrans Houttuyn	nutmeg and maze	seeds and arils	-
Petrosinellum crispum (Mill.) Nyman	parsley	roots and leaves	nutrient (root, leaf) oleoresin (seeds)
Pimenta dioica (L.) Merr.	pimento	leaves and berries	oleoresin (berries)
Pinus sp.	pine	needles and branches	oleoresins from wood (turpentine)
Syzygium aromaticum (L.) Merr. et Perry	clove	flower buds and leaves	oleoresin (buds)

The storage of essential oils in higher plants is not restricted to specialized plant parts. EOs occur in both roots, stems, leaves, flowers and seeds, or in the plant as a whole. Both epidermal or mesophyll tissue can function as the site of terpene biosynthesis in general, whereas typical storage cells or cell structures characterize the taxonomic group of aromatic plants: Oil cells, secretory glands, ducts and canals and the *Labiatae*-typical glandular trichomes (*capitate* and *peltate glands*). Depending on morphological structures, varying secondary metabolism and thus, determined signalling and defence functions of EOs in plant organs, the pronounced alteration gives the ability to obtain essential oil qualities that are quite different from one and the same plant. Some examples of multi-purpose aromatic herbs, spices and medicinal plants are given in Table 2-1.

One has to make a distinction between the tools and techniques used for the production and those for the analysis of EOs. Although the technical equipment for distillation, extraction and pressing is used for both purposes, the aim establishes the difference. Industrial-scale EO extraction focuses on mass production whilst laboratory applications often work on a micro-scale in order to isolate small quantities within a short time for qualitative and quantitative analyses. EOs are per definition produced from raw plant material by distillation or pressing (Table 2-2). Strictly speaking, EOs that are isolated by the use of solvents, sub- or supercritical CO_2 extraction are not genuine essential oils since non-volatile and waxy components have to be removed by fractionation (Lawrence, 2002).

Table 2-2: Classical techniques for high-yield production of EOs and aromatic extracts.

Product	**Technique**	
Essential Oil	Distillation	steam distillation hydrodistillation
	Pressing	hot cold
Aromatic Extract	Solvent Extraction	resins, concrètes, absolues
	Enfleurage	pomades, absolues
	CO_2-extraction	resins, aromatic essences
	Alcoholic Extraction	tinctures

2.3 Factors affecting EO Quality

Since often only one or a few components are responsible for the desired effect or application, importance has been attached to the characterization and analysis of EOs in recent decades. Besides cosmetics, perfumes and flavouring, EOs have been studied with regard to their antimicrobial (Hirasa and Takemasa, 1998; Cowan, 1999) and antioxidant properties (Hirasa and Takemasa, 1998; Stashenko et al., 2002). Several terpenes are known to be important mediators in plant-insect-interactions

(Harrewijn et al., 2001) and their capability for biological control for pest and disease management in plant production is an area of growing interest (e.g. Isman, 2000; Quintanilla, 2002; Quintanilla et al., 2002). EOs show physiological (Hirasa and Takemasa, 1998), anti-tubercular (Cantrell et al., 2001) and anti-tumour effects (Loza-Tavera, 1999). Their biosynthetic production through biotechnological applications (Deans and Svoboda, 1993; Hsu and Yang, 1996; Nishimura and Noma, 1996; Kribii et al., 1999; Barkovich and Liao, 2001; Nagata and Ebizuka, 2002) and synthesis of new terpenoids (Gautschi et al., 2001) have been investigated.

Despite the utilization of terpenoids in biomedical, agricultural and biotechnological applications, Sections 2.3 and 2.4 and particularly, Chapter 4, will focus on essential oil quality and variability of oil composition. Although EOs may be produced from an endemic population, there can be several reasons why the composition and thus, the EO quality from aromatic plants might differ greatly. Genetic, physiological and environmental factors as well as processing conditions, which are presented in Table 2-3, may play an important role (Hay, 1993; Hay and Svoboda, 1993; Rohloff, 1999 and 2002a; Rohloff et al., 2000a and 2002c; Lawrence, 2002).

Table 2-3: Factors influencing the composition of commercial EOs.

Factor		Description
Genetics	Taxon	clone, hybrid, cultivar, population
	Infraspecific	chemotypes (distinct populations within a species)
Physiology	Ontogenetic	developmental changes (vegetative <> generative)
	Plant organ	morphological differences (root, leaf, flower, seed)
Environment	Climate	light, temperature, edaphic factors
	Origin	latitude, height above MSL, country, continent
	Agriculture	cultivation technique, fertilizer, irrigation, harvest time
Processing	EO isolation	distillation, extraction, maceration, pressing, enfleurage
	Storage	effects of aging, ΔT, rH etc. on raw material or EO
	Adulteration/ Standardization	EO blending prior to distillation/ directly into the EO

Infraspecific and intervarietal differences can be observed in both morphology and chemical structures, which establish the basis for determining important chemically defined populations or *chemotypes* (Hay and Svoboda, 1993). Despite aromatherapeutic demands, which require that the medicinal value of an essential oil be based on its complete composition rather than its constituent parts (Franchomme et al., 1990), one still has to consider the chemical specification of EOs with regard to toxic concentrations of single constituents (Tisserand and Balacs, 1995; Lawless, 1996; Price and Price, 1999). Table 2-4 gives an overview about the main chemotypes of important aromatic herbs.

Table 2-4: Chemotypical variation of some economically important aromatic herbs and spices, updated after Boelens (1991), Franz (1993) and Lawless (1996).

Botanical name	English name	Main chemotype
Achillea millefolium L.	yarrow	(pro)azulene, azulene-free, caryophyllene, germacrene D, farnesene
Artemisia vulgaris L.	common wormwood	α-/β-thujone, 1,8-cineole, linalool camphor
Anethum graveolens L.	dill	carvone, limonene, phellandrene
Carum carvi L.	caraway	carvone, limonene
Chamomilla recutita L.	chamomile	bisabolol, bisabololoxide A/ B, bisabolonoxide A
Cuminum cyminum L.	cumin	cumin aldehyde, pinene
Foeniculum vulgare L. var. *dulce*	sweet fennel	anethole, fenchone, limonene, estragole
Lavandula angustifolia Mill.	lavender	linalool, linalyl acetate
Melissa officinalis L.	lemon balm	geraniol, citronellol
Mentha × *piperita* L.	peppermint	menthol, carvone, limonene, linalool
Mentha spicata L.	spearmint	carvone, menthone, piperitone
Ocimum basilicum L.	sweet basil	estragole, linalool, eugenol camphor, methyl cinnamate
Origanum majorana L.	marjoram	terpineol, sabinene hydrate
Origanum vulgare L.	oregano	carvacrol, thymol
Rosmarinum officinalis L.	rosemary	verbenone, 1,8-cineole, α-pinene
Salvia officinalis L.	Dalmatian sage	α-/β-thujone, thujone-free, 1,8-cineole, camphor
Thymus vulgaris L.	thyme	thymol, carvacrol, linalool, geraniol, thujanol, terpineol
Valeriana officinalis L.	valerian	valeranone, valeranal, cryptofauronol

Not only ontogenesis but especially infraspecific variation, i.e. differences between individuals from identical species, has to be taken into account for the use of appropriate numbers of plant samples when determining EO quality regarding their terpene and phenylpropene composition. There is not even a correlation between morphological and chemical characters (Franz, 1993), which makes it difficult and intricate to characterize chemotypes on a phenotypic basis. The influence of environmental factors on chemotypic differentiation has been reported widely. Depending on different light conditions and temperatures, Massoud (1989) reported varying oil yield and expression of bisabolol and chamazulene in chamomile, and Franz (1993) described the geographical distribution of chamomile chemotypes throughout Mediterranean regions. When viewed on a microscale, temperature and light condition changes during a period of a day might be responsible for distinct variations of EO yield and composition as well (Fahlen et al., 1997; Silva et al., 1999; Rodrigues et al., 2002). Taking newer scientific reports on coriander (*Coriandrum sativum* L.) as an example and starting point, one might explore the whole complexity of questions related to EO composition, biosynthesis, production, quality, analysis and processing (Table 2-5).

Table 2-5: Various analytical approaches and applications regarding the EO composition of coriander (*Coriandrum sativum* L.).

Genotype	*Evaluation of coriander (Coriandrum sativum) collection for essential oil* – Kalra et al. 1999
Origin	*Compositional differences of coriander fruit oils from various origins* – Pino et al. 1993
Cultivation	*Coriander essential oil composition from two genotypes grown in different environmental conditions* – Gil et al. 2002
Plant organ	*Essential oil composition of cilantro* – Potter 1996
Ontogenesis	*Commercial essential oils: truths and consequences* – Lawrence 2002
Symbiosis	*Mycorrhization of coriander (Coriandrum sativum L.) to enhance the concentration and quality of essential oil* – Kapoor et al. 2002
Production	*Coriander spice oil: Effects of fruit crushing and distillation time on yield and composition* – Smallfield et al. 2001
Biotechnology	*Production of volatile compounds in suspension cell cultures of Coriandrum sativum L. and Levisticum officinale Koch* – Hsu & Yang 1996
Analysis	*Extraction of coriander seed oil by CO_2 and propane at super- and subcritical conditions* – Illes et al. 2000
Storage	*Influence of the duration and conditions of storage on the composition of the essential oil from coriander seeds* – Misharina 2001
Drying	*Microwave drying of selected greens and their sensory characteristics* – Fathima et al. 2001
Adulteration	*GC-IRMS in the authenticity control of the essential oil of Coriandrum sativum* – Frank et al. 1995
Olfaction	*Sedative effect on humans of inhalation of essential oil of linalool: Sensory evaluation and physiological measurements using optically active linalools* – Sugawara et al. 1998
Pharmacology	*Coriandrum sativum - effect on lipid metabolism in 1,2-dimethyl hydrazine induced colon cancer* – Chithra & Leelamma 2000
Preservation	*Evaluation of antilisterial action of cilantro oil on vacuum packed ham* – Gill et al. 2002

The idea of "essential oils as a pure, natural product from raw plant materials" aquires another dimension when reading Lawrence's article (2002) about the production and *standardization* of commercial EOs. Standardization is carried out by blending EOs in order to improve composition consistency of the oils and to minimize seasonal and geographical variations. On the other hand, *adulteration*, through the addition of synthetic components, oil fractions or oils or even other, "foreign" natural products, is carried out for financial gain. In any case, the genuineness and character of the EO is reduced with regard to olfaction, antioxidant properties and biomedical applicability. Thus, when demanding pure, high-quality EOs, the use of advanced analytical tools for revealing the source authenticity and possible adulteration, is inevitable.

2.4 "Norwegian Herb Production (NUP)" 1994-1998

As part of the project "Norwegian Herb Production (NUP)" in the period between 1994-1998, research has been carried out at the author's laboratory at The Plant Biocentre on essential oil yield and composition of the following aromatic herbs: chamomile (*Chamomilla recutita* L.), lemon balm (*Melissa officinalis* L.), oregano (*Origanum vulgare* L.), peppermint (*Mentha x piperita* L.), sachalinmint (*Mentha sachalinensis* Briq. (Kudô)), thyme (*Thymus vulgaris* L.) and yarrow (*Achillea millefolium* L.). The study was partly financed by the Norwegian Research Council (NFR) and addressed to give a better understanding of cultivation and processing needs with regard to *fertilization*, *winter hardiness*, *dry matter, EO yield* and *EO quality* depending on *harvest time* and *genotype* of the selected herbs and cultivars. The results presented have not been published before in scientific media, and will be used to discuss the influence of environmental factors as already mentioned in Chapter 2.3, *Factors affecting EO Quality*.

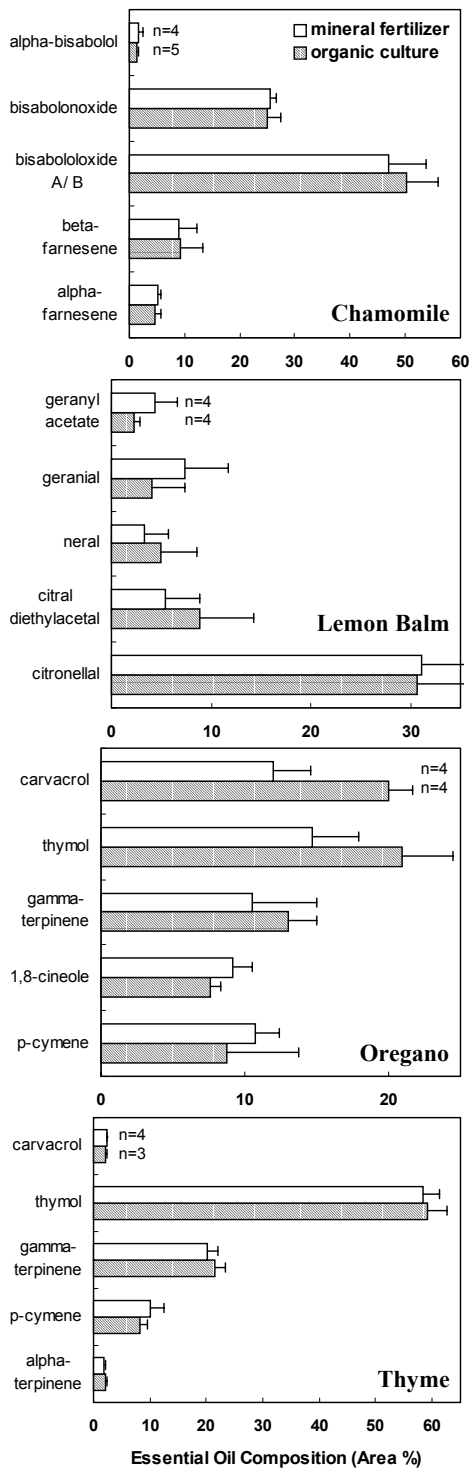


Figure 2-3: EO composition of the aromatic herbs chamomile, lemon balm, oregano and thyme, grown in Norway in 1994 (NUP-project) with regard to fertilization. Horizontal bars indicate standard deviation of average values from all trial fields.

Source: Iversen et al., 1994a and 1994b

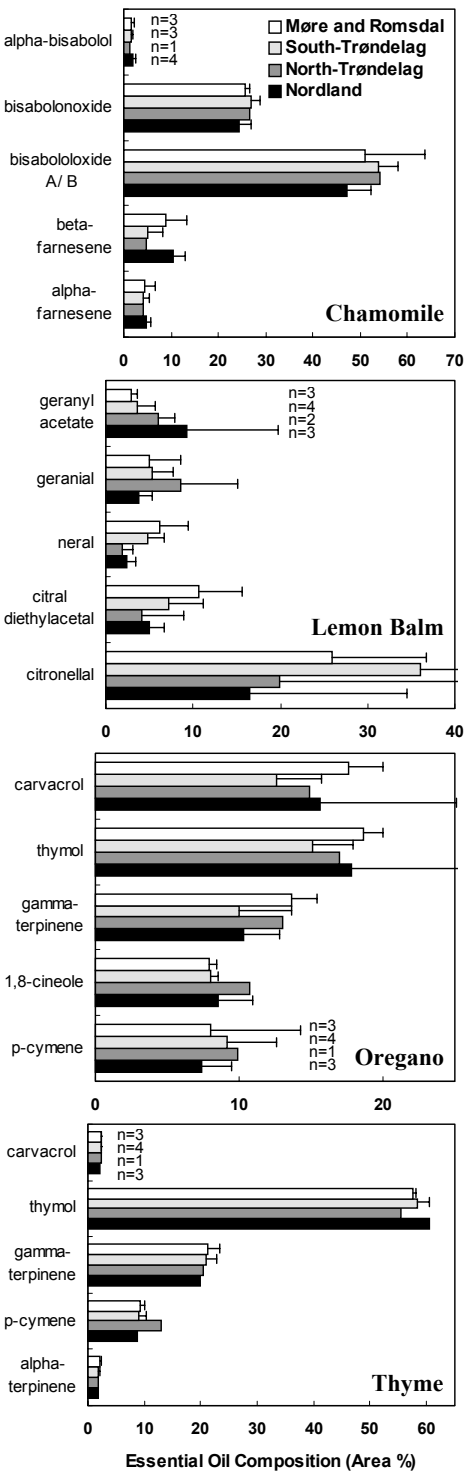


Figure 2-4: EO composition of the aromatic herbs chamomile, lemon balm, oregano and thyme, grown in Norway in 1994 (NUP-project) with regard to geographic localityof the trial fields. Horizontal bars indicate standard deviation of average values from all field trials.

Source: Iversen et al., 1994a and 1994b

Figures 2-3 and 2-4 represent results from the pre-project period in 1994 and show the essential oil composition of four different herbs with regard to fertilizer management and geo-graphic locality of the trial fields. The trials were carried out in four counties: Møre- and Romsdal in the southern region of central Norway at 62º-63ºN, South-Trøndelag at 63º-65ºN, North-Trøndelag at 64º-65ºN and Nordland in northern Norway at a latitude between 65º-69ºN. The EO was obtained by hydrodistillation from dried plants and analysed by GC-FID (oregano, thyme) and GC-MS (chamomile, lemon balm).

Using fertilizer use for comparison (Figure 2-3), high variation was observed when averaging the results from the different counties, and no distinct trends could be found with regard to differing light conditions, day length and/or temperature effects. Data on thyme and oregano were most consistent and showed the lowest standard deviation. Both species belong to the Labiateae family and are closely related to each other with regard to the occurrence of the terpenoid phenols thymol and carvacrol, which were increased under organic culture. In general, the chemotypic distribution of the main-character terpenes of the studied herbs was only slightly affected,

although tendencies of environment-dependant increased or decreased concentration of terpenes could be observed (Table 2-4: lemon balm and thyme). The effect of differing light conditions was not confirmed by more detailed studies on two peppermint clones in the trial period 1995-96 (Rohloff et al., 2000b), in contrast to studies on *Mentha* species by Voirin and co-workers (1990) and Fahlen and co-authors (1997) reporting altered terpene biosynthesis as an effect of photoperiod and day length. EO yield seemed to be decreased in the northern county, Nordland, compared to the trial fields in central Norway except for data on oregano, which is in accordance with studies on peppermint (Rohloff et al., 2000b), whereas the effect of fertilization was not distinct enough (see Table 2-6).

Table 2-6: EO production of the aromatic herbs chamomile, lemon balm, oregano and thyme, grown in Norway in 1994 (NUP-project) with regard to fertilization and geographic locality of the trial fields.

Plant Species:		**Chamomile**	**Lemon Balm**	**Oregano**	**Thyme**
Mineral Fertilizer	(n=3/4)	0.63	0.14	0.37	2.91
Organic Culture	(n=4/5)	0.56	0.15	0.49	2.60
Møre- and Romsdal	(n=2/3)	0.60	0.18	0.48	2.97
South-Trøndelag	(n=3/4)	0.63	0.15	0.36	2.86
North-Trøndelag	(n=1/2)	0.60	0.10	0.37	3.20
Nordland	(n=3/4)	0.53	0.08	0.50	2.17

Source: Iversen and Steen, 1996 and 1997

Based on the results from the pre-project, the NUP-project was extended by more field trials during the period 1995-96 covering the Norwegian latitudes from 59°N to 67°N. In addition, the herbs sachalinmint (variety 'Mentolcsepp') and yarrow were introduced in the project. The main task was to study the EO yield and composition of six aromatic herbs with focus on developmental stage and optimal harvest time (Table 2-7).

Table 2-7: EO production (in %) of six aromatic herbs grown in Norway during the period 1995-96 (NUP-project) with regard to ontogenetic effects and harvest time.

Growth stage:		**vegetative**	**early bloom**	**full bloom**
Lemon Balm		0.12	0.13	0.08
Oregano		0.40	0.55	0.53
Peppermint	clone **'**Hardanger'	1.65	1.88	2.40
Peppermint	clone 'Tøyen'	1.60	1.65	1.85
Sachalinmint	cv. 'Mentolcsepp'	2.47	3.09	2.68
Thyme		2.95	3.31	3.20
Yarrow		0.14	0.13	0.34

Source: Iversen and Steen, 1996 and 1997

With the exception of lemon balm, the EO yield was lowest in the vegetative growth stage. Peppermint and yarrow showed high oil production in full bloom, whereas observations for the other herbs indicated that early flowering would be the optimal time for harvest. Table 2-8 shows the complexity and importance of ontogenetic effects on

selected terpenes and EO quality in general. The menthol content of mint species, e.g., increased dramatically from the vegetative stage to full flowering, i.e. the plants should be harvested in full bloom to obtain EOs with a desirable high menthol and low menthone content. On the other hand, high concentrations of the undesirable menthofuran in mint flowers compared to leaf oil (Rohloff, 1999), favour an earlier harvesting.

Table 2-8: Characteristic terpenes of six aromatic herbs grown in Norway during the period 1995-96 (NUP-project) with regard to ontogenetic effects and harvest time.

Species	Terpene	vegetative	early bloom	full bloom
Lemon Balm	**citronellal**	19.84	29.43	23.19
	neral	17.27	17.53	15.86
	geranial	25.99	24.14	24.29
Oregano	**γ-terpinene**	11.47	10.05	4.27
	thymol	8.23	6.60	5.07
	carvacrol	6.43	4.60	1.50
Peppermint cv. **'**Hardanger'	**1,8-cineole**	5.24	5.32	5.20
	menthone	30.60	31.06	19.65
	menthol	39.34	44.85	55.15
Peppermint cv. 'Tøyen'	**1,8-cineole**	4.30	5.93	5.50
	menthone	43.28	27.60	19.60
	menthol	37.03	46.50	54.45
Sachalinmint	**menthone**	14.36	12.04	10.30
	isomenthone	4.23	4.36	3.95
	menthol	74.52	78.30	83.58
Thyme	**γ-terpinene**	19.79	19.57	13.94
	thymol	50.05	57.23	66.60
	carvacrol	2.14	2.30	7.47
Yarrow	**β-pinene**	5.15	2.20	16.19
	1,8-cineole	5.80	4.23	5.97
	α-farnesene	8.19	10.12	2.09

Source: Iversen and Steen, 1996 and 1997

Comparable trends could be found for lemon balm (citronellal), thyme (thymol, carvacrol) and the complex oil of yarrow (β-pinene) (see Rohloff et al., 2000a), while a decreasing effect on phenol concentrations was observed for oregano. Lawrence (2002) reports similar ontogenetic effects with regard to terpene distribution, presenting examples on *Tagetes minuta* and *Coriandrum sativum* and simultaneously pointing out that environmental effects might be more pronounced in temperate and sub-tropical zones. Again, the summarised results from the NUP-project underscore the importance of knowledge and experience from plant research studies, for the elaboration of plant grower guidelines for the cultivation of aromatic plants (Dragland and Galambosi, 1996; Iversen and Rohloff, 1998). On the background of SPME analysis, Chapter 3, *Essential Oil Research*, and Chapter 4, *HS-SPME Applications*, continue to outline the influence of physiological and environmental factors and the effect of processing on the EO volatile composition of plants and plant food products.

3. Essential Oil Research ✧ SOLID-PHASE MICROEXTRACTION

3.1 Devices and Procedures

Solid-phase microextraction (SPME) is a relatively new extraction technique for the isolation and analysis of chemical compounds of different origins and structures. Since its introduction by Belardi and Pawliszyn (1989) and further developments in the early 1990s (Arthur et al., 1992a and 1992b; Louch et al., 1992; Buchholz and Pawliszyn, 1993; Zhang and Pawliszyn, 1993), this technique has undergone a process of innovation due to new approaches and devices. The following section gives a brief overview about accessories and developments before stressing the question about SPME applications in modern food analysis and focussing on the determination and quality control of aromatic herbs and medicinal plants. To get a better idea of the techniques and applications mentioned, essential terms and expressions are underlined.

SPME is a solvent-free and non-destructive sample preparation technique, where the analytes are extracted from a solvent, fluid or solid partitioned in a sample/ air/ extraction fibre–system (*headspace* HS) or a sample/ extraction fibre–system (*direct immersion* DI). The SPME procedure is followed by gas or liquid chromatography or electrophoresis (Supelco, 2001). Due to the multifunctionality of the extraction process (HS, DI), most of the reported applications deal with GC analysis coupled with the most common detection methods (MS, FID, NPD, etc.), and only a few number of applications have been developed for LC, HPLC and other separation techniques. If not mentioned otherwise, the following section exclusively describes GC separations. GC-MS detection in total-ion-count (TIC) modus has been chiefly applied, but mass-selective ion monitoring (SIM) for the detection of structure-characteristic compounds has also been applied (e.g. Wang et al., 1997; Butzke et al., 1998; Curren and King, 2001; Moeller and Kraemert, 2002). Commonly, SPME injection is followed by chromatographic separation but newer concepts for the measurement of total volatile compounds of food by directly linking the injection port to the detector - flame ionisation detector FID (Azodanlou et al., 1999) or nitrogen phosphorus detector NPD and FID (Béné et al., 2001) - have also been investigated.

The analyte extraction is not exhaustive, i.e. small quantities down to ppt-levels can be detected by fibre absorption or adsorption (Camarasu et al., 1998; Lloyd et al., 1998; Miller and Stuart, 1999; Alpendurada, 2000; Curren and King, 2001; Ho and Hsieh, 2001; Holt, 2001) which allows multiple testing of identical samples if the initial sample concentration is high enough. The non-destructive character of SPME allows the monitoring of volatile compounds from living organisms such as plants (Kreuzwieser et al., 1999; Müller et al., 1999; Vercammen et al.,

2000; An et al., 2001; Zini et al., 2001; Flamini et al., 2002), *in vitro* cultures of plants (Alonzo et al., 2001; Maes et al., 2001), insects (Borg-Karlson and Mozuraitis, 1996; Auger et al., 1998; Jones and Oldham, 1999), microorganisms (Arnold and Senter, 1998; Demyttenaere et al., 2000; Demirci et al., 2001; Demyttenaere et al., 2001; Fravel et al., 2002), algae in surface water (Watson et al., 1999) and enzymatic processes (Jarvenpaa et al., 1998; Cass et al., 2000; Freire et al., 2001; Vianna and Ebeler, 2001; Mallouchos et al., 2002). The screening of volatile compounds from leaves and flowers of *Fragaria* by SHS-SPME sampling over a period of three days gave reliable results about metabolic changes and induced sesquiterpene production as an effect of wounding/ piercing of leaf and flower stems (Rohloff, 2002; unpublished results).

SPME offers also a promising tool for the control of quality and shelf life of fresh or processed food products with regard to ripening, aging and storage of fruits (Ibanez et al., 1998; Wan et al., 1999; Beaulieu and Grimm, 2001; Fallik et al., 2001; Holt, 2002; Koprivnjak et al., 2002; Lamikanra et al., 2002), must (Sala et al., 2000), cheese (Lecanu et al., 2002), oxidative reactions in milk (Marsili, 1999a and 1999b) and vegetable oils (Stashenko et al., 2002). The importance of terpene-containing grassland herbs for the quality of milk aroma has been investigated by Cornu and co-workers (2001). Off-flavour or off-odour effects as mentioned for dairy products play also an important role for other food products such as juice (Miller and Stuart, 1999; Rousseff and Cadwallader, 2001), seasoned spaghetti (Roberts and Pollien, 1997) or pharmaceutical products (Sides et al., 2001).

The sampling itself may be carried out in-laboratory or on-site as discussed by Pawliszyn (2001), e.g. Müller, Górecki and Pawliszyn (1999) described a new field SPME sampler equipped with a two-leaf closure for preservation of the analytes (organic solvents) and Namiesnik and co-workers (2000) described the analysis of atmospheric, indoor and workplace air. Gas-tight SPME sampling has been investigated, offering greater fibre sensitivity in HS applications (Camarasu et al., 1998). Ruiz and co-workers (2001) reported the development of a promising device that allows direct SPME sampling from solids without sample preparation through protection of the fibre in the core, in contrast to fibre-contact sampling reported by Verhoeven and co-workers (1997) and Grassi and co-authors (2002). A newer microextraction technique - in-tube SPME - offers the same advantages as fibre SPME, and is based on the extraction of analytes by using an open tubular fused-silica column and separation on HPLC or LC systems (Kataoka et al., 2000; Lord and Pawliszyn, 2000; Kataoka, 2002). The challenging problem about the extraction of polar compounds from biological matrices can be met by derivatization techniques (Matich, 1999; Lord and Pawliszyn, 2000; Mills and Walker, 2000) where the derivatization takes place in the sample matrix, on-fibre or in the GC injection port. SPME also allows for combining procedures with other sampling or extraction techniques, e.g. microwave-assisted extraction

MAE (MAE-SPME) coupled with GC (Roberts and Pollien, 1997; Wang et al., 1997; Ho and Hsieh, 2001) or HPLC analysis (Falqui-Cao et al., 2001), accelerated solvent extraction ASE (ASE-SPME) (Wennrich et al., 2001), steam distillation (SD-HS-SPME) (Page, 1999) and cryo-trapping of volatiles (Jaillais et al., 1999). The thermal desorption of analytes is normally carried out by manual fibre injection into the GC, but several applications dealing with automated SPME and injection for routine analyses for the detection of contaminants and quality control have been reported for both fibre (Arthur et al., 1992a; Penton, 1994; Butzke et al., 1998; Coleman et al., 1998; Coleman and Lawrence 2000; Pham-Tuan et al., 2001; Zini et al., 2002) and in-tube SPME applications (Kataoka, 2002). Elmore and co-workers (2001) describe the potentials of two-fibre SPME by using two different coatings and simultaneous GC injection.

Apart from the applicability and flexibility of the described analytical approaches, the performance of the SPME procedure depends undoubtedly on strict control of the parameters of the chosen method to obtain accurate and repeatable results.

3.2 Extraction Parameters

Many factors are responsible for the efficiency of the extraction process when carrying out HS-SPME coupled with GC analysis: *fibre coating* and *selectivity*, *sample matrix* and *size*, *HS volume*, *pH, salt concentration* and *agitation* (Matich, 1999; Kataoka et al., 2000). *Temperature* effects and *extraction time* will be discussed in detail in concerning questions about equilibrium time, concentration and analyte linearity in Chapter 4.2. The choice of polarity of the fibre type is especially crucial for an optimised SPME procedure with regard to the chemical and physical properties of the analytes being extracted. Polydimethylsiloxane (PDMS), divinylbenzene (DVB), carboxene (CAR), carbowax (CW) and polyacrylate (PA) are the most common fibre coatings. PDMS was the first commercially accessible type that was widely applied for non-polar analytes, whereas coatings such as DVB, CW and PA are recommended for polar compounds and general use as shown for PA (e.g. Chin et al., 1996; Vercammen et al., 2000). CAR coating shows good applicability for the extraction of gaseous and highly volatiles and trace compounds. About 30 different fibre types and combinations of coatings are accessible in order to cover a broad range of analyte polarities. Many authors point out that combined fibre coatings offer greater advantages for the extraction of volatiles from complex matrices (Miller and Stuart, 1999; Hill and Smith, 2000; Jelen et al., 2000; Curren and King, 2001; Elmore et al., 2001) and mention the better storage capacity of combined coatings (Müller et al., 1999). Bicchi and co-workers (2000b) investigated the influence of fibre coating in HS-SPME-GC analyses of aromatic and medicinal plants and emphasized the use of combined coatings of non-polar–polar components. Disadvantages of the fibre coatings used might be

artefact formation (Verhoeven et al., 1997; Baltussen et al., 2002) and characteristic background signals (*bleeding*) of cyclic siloxanes from PDMS, PA degradation products and phtalates as investigated by Vercammen and co-workers (2000) and phtalates and benzene derivates from PDMS/DVB and siloxanes from pure PDMS fibres as observed by the present author (unpublished results). In addition, the individual fibre characteristics and extraction performance may differ from batch to batch, which makes fibre optimisation and method testing inevitable for continuous sampling.

SPME fibres differ not only in the polarity of the stationary phase, but also in the thickness of the fibre. Pure PDMS coatings e.g., can be obtained with 100, 30 or 7 µm film thickness, thus influencing the amount of extracted analytes. Other factors such as extraction time, temperature conditions and sample concentration play an important role as well. Questions related to DI vs. HS sampling have already been emphasised by Yang and Peppard (1994), underscoring the suitability of HS-SPME for more volatile compounds. On the other hand, DI-SPME is more recommendable for the sampling of less-volatile compounds, but non-volatile compounds remaining on the fibre, thus affecting reproducibility, fibre lifetime and detection background, may be disadvantageous (Kataoka et al., 2000; Lord and Paliszyn, 2000). Since the sample matrix in normal cases consists of a mixture of compounds with varying polarity, boiling point and molecular weight, the percentage contribution of analytes on-fibre might reflect quite differing component distributions depending on extraction time under the given sampling condition. Varying analyte affinity towards solid sorbents (Lord and Pawliszyn, 2000) or non-polar coatings (Rohloff, 2002a) can result in displacement effects such as a factor of time. Sample weight might also affect the competition of compounds towards the fibre, especially when the sample volume is very small (with a relatively higher headspace volume and vice versa), i.e. the headspace concentration of analytes is significantly decreased by SPME (Kovačevič and Kač, 2001). Therefore, calibration methods are indispensable when it comes to quantification. The use of external or internal standards, isotopic labelling or standard-addition through re-analysis has been discussed by Lord and Pawliszyn (2000). Freire and co-authors (2001) point out the greater advantages of the internal standard method compared to standard addition, which was applied by Shang and co-workers (2002) and minimized errors with regard to the extraction and injection process. External calibration has also been applied (Buszewski and Ligor, 2001), although this option might only be useful when studying simple matrices with low variability.

SPME shows high sensitivity towards volatile organic compounds compared with classical headspace techniques such as SHS, DHS, purge-and-trap, direct thermal desorption (DTD) and gas-tight sampling. Many authors point out the strength of SPME for qualitative analyses (Coleman and Lawrence, 1997) and its applicability for the extraction of less volatile

and/ or polar compounds (Matich et al., 1996; Miller and Stuart, 1999; Bicchi et al., 2000b; Vercammen et al., 2000), although SPME might discriminate highly volatile and/ or trace compounds (Arnault et al., 2000; Vercammen et al., 2000). Besides a rise in temperature for the induction of increased diffusion coefficients and head space concentrations of analytes and decreased equilibrium time (Lord and Pawliszyn, 2000), one might change the ionic strength of liquid samples, with e.g. sodium chloride (NaCl) to increase the sensitivity of SPME (Harmon, 1997). The salting-out effect can improve the recovery of the aroma impact compound furaneol (2,5-dimethyl-4-hydroxy-3(2*H*)-furanone) from strawberries which is difficult to extract by common HS-SPME (Ulrich et al., 1995), when using 25 % NACl (w/v) in strawberry juice samples (Rohloff et al., 2002b). In general, liquid samples should be agitated by continuous stirring throughout the equilibrium and extraction period to reduce equilibration time (Matich, 1999) and to improve accuracy and method standardization in routine laboratory work (Holt, 2001). When comparing HS-SPME of *Fragaria* samples from cut strawberries or juice, one might prefer liquid instead of solid phases because of the possibility that magnetic stirring will result in shorter analysis time and better reproducibility (Rohloff, 2001; unpublished results). The volatility of analytes can also be positively affected by adjustment of the pH of liquid samples depending on whether one whishes to extract acidic (pH ↓) or basic analytes (pH↑) (Kataoka et al., 2000). In fact, in the case of HS-SPME analysis of solid samples such as dried or processed plant products, the choice of optimal fibre type, equilibrium and extraction temperature and time plays a crucial role.

3.3 SPME Detection of EO Volatiles in Plants and Food Products

SPME can be utilized for various applications and approaches in food analysis, and quality control is one of the key words. Although this technique allows for the analysis of less volatile or thermal labile compounds coupled with liquid chromatography (LC), most all the methods have been developed based on the DI- and HS-SPME of food volatile compounds and contaminants coupled with gas chromatography (GC). Applications of solid-phase microextraction within environmental and biomedical/ pharmaceutical research have been reported and reviewed elsewhere and will not be discussed here (*environmental*: Chai and Pawliszyn, 1995; Nilsson et al., 1995; Sedlakova et al., 1998; Page, 1999; Page and Lacroix, 2000; Alpendurada, 2000; Moeder et al, 2000; Beltran et al., 2000; Buszewski and Ligor, 2002; *biomedical*: Lord and Pawliszyn, 2000; Mills and Walker, 2000; Snow, 2000; Theodoridis et al., 2000; Ulrich, 2000; Pawliszyn, 2001). The following table summarizes the different kinds of applications for plant and food analysis (Table 3-1), which have been exhaustively discussed and reviewed (Harmon, 1997; Braggins et al., 1999; Matich, 1999; Pawliszyn, 1999; Kataoka et al., 2000; Sides et al., 2000; Rouseff and Cadwallader, 2001; Supelco, 2001).

Table 3-1: SPME applications for the detection and analysis of flavour and aroma volatiles from plants, plant products and food in general.

SPME application	Important Analytes
Vegetables and Fruits	
apple, strawberry, tomato, kiwi, melon, etc.	- *hydrocarbons, alcohols, acids, esters, ketones, aldehydes, terpenes and pesticides*
onion, truffle, vegetables in general	- *hydrocarbons, alcohols, acids, esters, S-containing volatiles and pesticides*
herbs, spices, medicinal plants	- *terpenes, phenylpropenes, phenols, hydrocarbons and esters*
tobacco	- *aldehydes, terpenes and phenols*
vegetable oils	- *fatty acids, alcohols, aldehydes, ketones and VOC contaminants*
honey	- *hydrocarbons, alcohols, acids, ketones, aldehydes, terpenes and pesticides*
Juices and Soft drinks	
orange, tomato, strawberry etc.	- *alcohols, acids, esters, ketones, aldehydes and terpenes*
must	- *pyrazines and S-containing volatiles*
coffee	- *pyrazines and pyridines*
Alcoholic beverages	
wine	- *alcohols, acids, esters, terpenes, S-containing volatiles (isothiocyanates, thiazoles), phenols and pesticides*
vodka, sake, whiskey, etc.	- *esters, alcohols and fatty acids*
beer	- *alcohols, esters, S-containing volatiles and terpenes*
Dairy products	
cheese	- *fatty acids and lactones*
milk	- *fatty acids and aldehydes*
whey protein	- *fatty acids and diacetyl*
Living plants	
flowers	- *alcohols, esters, aldehydes, terpenes and S-containing volatiles*
leaves	- *alcohols, esters, acids, aldehydes, terpenes and phenylpropenes*

Since the main constituents of EOs – monoterpenes, sesquiterpenes and phenylpropenes – partly show high volatility, it is not surprising that a number of (almost 50) plant species or species groups have been investigated by SPME-GC techniques (Table 3-2).

Table 3-2: SPME applications for different plant sample matrices for the study of EO volatiles with regard to biosynthesis, chemotaxonomy and quality control.

Plant species	Matrix	Application	Reference
anise	drops	quality control	Czerwinski et al., 1996
arnica	root, leaf, flower	terpene screening ontogenetic variation	Rohloff,1999* [Fig. 4-6, Table 4-5]
bay tree	leaf	terpene screening	Diaz-Maroto et al., 2002b
black pepper	fruit	terpene screening	Harmon 1997
brown boronia	flower	volatile monitoring	MacTavish et al., 2000
buchu	stem	terpene biosynthesis enantiomeric analysis	Fuchs et al., 2001c
cedar	EO	terpene screening quality control	Coleman & Lawrence,1997 Coleman et al., 1998 Coleman&Lawrence, 2000
chamomile	flower	terpene screening	Rohloff, 1998* [Table 4-1]
cinnamon sp.	bark	species identification	Miller et al., 1996
	bark	terpene screening	Yang & Peppard, 1995
costmary	EO essences	terpene screening quality control	Gallori et al., 2001
curry	powder	terpene screening	Harmon, 1997
dill	herb	terpene screening	Rohloff, 1998* [Table 4-1, Figure 4-1 and 4-2] and
	herb	variety fingerprinting	Rohloff, 1998* [Figure 4-5]
echinacea sp.	herbal remedy	terpene screening quality control	Rohloff, 1999* [Table 4-8]
eucalyptus sp.	leaf	leaf volatile monitoring	Zini et al., 2001
	leaf	terpene screening	Zini et al., 2002
	leaf	leaf volatile monitoring	Wirthensohn et al., 2000
fennel	seed	variety fingerprinting	Krüger & Zeiger, 1996
Fraser fir	leaf	terpene screening	Vereen et al., 2000
geranium sp.	herb	terpene biosynthesis enantiomeric analysis	Wust et al., 1996
	herb	terpene biosynthesis enantiomeric analysis	Fuchs et al., 2001b
ginger	EO	terpene screening	Supelco, 1998
gourd sp.	flower	terpene screening	Fernando & Grun, 2001
grassland	herb	plant society volatiles	Cornu et al., 2001
guava	fruit	flavour screening	Paniandy et al., 2000
hops	cones	variety fingerprinting	Field et al., 1996; Kač & Kovačevič, 2000 Kovačevič & Kač, 2001 Rohloff, 2001* [Table 4-2]
	cones	terpene screening	Kenny, 2000
jasmine sp.	plant	terpene screening in-vivo study	Vercammen et al., 2000
lavender	flower	terpene screening in-vivo study	An et al., 2001
lemon	EO	terpene screening	Supelco, 1998
moss	leaf	terpene screening	Saglam et al., 2001
oregano	herb	terpene screening	Diaz-Maroto et al., 2002b
osage orange	fruit	terpene screening	Peterson et al., 2002
peppermint	leaf	terpene screening	Rohloff, 1999 [Table 4-3]
	flower	ontogenetic variation	
	leaf,flower	terpene screening	Rohloff, 1998* [Table 4-1]

peppermint	herb	detection menthol	Ligor & Buszewski, 1999
	leaf	terpene biosynthesis enantiomeric analysis	Fuchs et al., 1999a, 1999b, 2000, 2001a
mint EO	EO	quality control	Coleman et al., 2002
	EO	terpene screening enantiomeric analysis	Coleman & Lawrence, 2000
mint EO in food products	candies, drops, etc.	menthol - menthone quality control	Ligor & Buszewski, 1999
	drops	quality control	Czerwinski et al., 1996
	menthol	quality control	Coleman & Lawson, 1998
	chocolate	quality control	Supelco, 1998
	chew.gum	quality control	Supelco, 1998
phlox sp.	bud flower	ontogenetic variation volatile monitoring	Irwin & Dorsett, 2002
pine sp.	leaf	terpene screening species identification	Schäfer et al., 1995
	wood, bark	terpene volatile monitoring	Flechtmann et al., 1999
rosemary	herb	terpene screening	Bicchi et al., 2000a+b
	EO	terpene screening enantiomeric analysis	Coleman & Lawrence, 2000
rose root	rhizome root	terpene screening	Rohloff, 2002b Rohloff et al., 2002c
sachalinmint	leaf and flower	ontogenetic variation	Rohloff et al., 2002a [Fig. 4-3, Table 4-4]
	leaf	method development	Rohloff, 2000* [Fig. 4-4]
sage	herb	terpene screening	Bicchi et al., 2000a+b
	leaf	wounding physiology	Zabaras & Wyllie, 2001
spearmint	EO	quality control	Supelco, 1998
	EO	terpene screening enantiomeric analysis	Coleman & Lawrence, 2000
spearmint EO in food products	chew.gum	quality control	Supelco, 1998
spruce	bark	terpene screening damage physiology	Pettersson, 2001
strawberry	leaf flower	volatile monitoring in-vivo study	Rohloff, 2002*
sweet basil	leaf	terpene screening quality control	Rohloff, 1998* [Table 4-1]
	leaf	terpene screening	Rohloff, 1997* [Table 4-6]
	leaf	terpene screening	Diaz-Maroto et al., 2002b
thyme		terpene screening	Bicchi et al., 2000a+b
tobacco	leaf	variety fingerprinting	Clark & Bunch, 1998
valerian	root	terpene screening	Bicchi et al., 2000a+b
viola sp.	flower	terpene screening in-vivo study	Flamini et al., 2002
yarrow	leaf flower	ontogenetic variation	Rohloff et al., 2000a

* unpublished results

HS-SPME is not only a proven tool for EO volatile screening and quality assessment of food products, but has also the advantage of being a technique for the monitoring of biological processes such as biosynthesis of secondary metabolites, ontogenesis and ripening and post harvest changes on the other hand, which are related to storage and processing of

plant products (Table 3-2). Biosynthetic studies dealing with the enantiomeric distribution of certain monoterpene hydrocarbons have especially attracted attention in the past 5 years. Since chiral compounds in EOs occur with characteristic enantiomeric patterns due to the enzymatically controlled biosynthesis (Lawrence, 2002) and due to the fact that their flavour properties are directly linked to their optical activity, SPME might be applied for the control of aroma impact volatiles in food (Ebeler et al., 2001), thus, facilitating EO volatile analysis with regard to plant source determination (Wust and Mosandl, 1999; Coleman and Lawrence, 2000) and the adulteration of EOs. The work of Fuchs and co-authors has focussed on monoterpene biogenesis from the *p*-menthane group in different plant sources (Fuchs et al., 1999a and 1999b; Fuchs et al., 2000; Fuchs et al., 2001a, 2001b and 2001c), but also monoterpene-ethers such as rose oxide (Wust et al., 1996) and microbial transformations have been studied (Demirci et al., 2001). The occurrence of EO volatiles in plants is not exclusively linked to aromatic herbs. Monoterpenes such as α- and β-pinene, β-myrcene, limonene, α- and β-phellandrene and sesquiterpenes, such as α- and β-caryophyllene, just to mention a few, are widely distributed throughout the plant kingdom and especially in conifers.

Many berry and fruit-bearing species are known for their relatively high contents of important monoterpene alcohols: linalool, citronellol, nerol, geraniol and nerolidol. These compounds along with other monoterpenes and hydrocarbon esters might be used as *markers* for wine flavour analysis (Penton, 1995; Muranyi and Kovacs, 2000; Buszewski and Ligor, 2001; Freire et al., 2001; Rocha et al., 2001; Schneider et al., 2001), the varietal classification of wines (Garcia et al., 1997, 1998a and 1998b; Pozo-Bayon et al., 2001), tobacco (Clark and Bunch, 1997 and 1998) and comparative studies on similarities between wines from grapes and lychee (Ong and Acree, 1999). The occurrence of linalool in strawberry cultivars such as 'Bounty', 'Senga Sengana' and 'Jonsok' can be the basis for variety studies (Holt, 2002) as well as for the quality control of strawberry juice after storage (Siegmund et al., 2001). The general composition and concentrations of monoterpenes, hydrocarbons (acids, esters etc.) and other aroma volatiles in tomato (Ulrich et al., 1997; Servili et al., 2000), orange juice (Steffen and Pawliszyn, 1996; Jia et al., 1998; Fan and Gates, 2001; Rouseff et al., 2001), mango (Shang et al., 2002) and Brazilian fruits (Augusto et al., 2000) might be the starting point for quality assessment with regard to aging/processing. The detection of plant-derived terpenes by SPME is not only restricted to aromatic plants as a biosynthetic source. Kohlert and co-authors (2002) describe an interesting application dealing with pharmacodynamic activity by tracing *thymol* in humans after oral application through the SPME analyses of plasma and urine. In this context, the next chapter will focus solely on applications and investigations dealing with the volatile composition and quality of aromatic herbs and medicinal plants.

4. HS-SPME Applications ✧ QUALITY OF AROMATIC HERBS

As described earlier, solid-phase microextraction offers a very fast, handy, reliable and inexpensive extraction tool for organic volatiles when coupling with gas chromatography analysis. Seen from a plant biological point of view, SPME shows high applicability to the detection of both terpenic, phenylpropene, aliphatic and sulphur-containing volatiles from various plant sources. *Plant physiology*, *chemotaxonomy* and *post harvest* are just some keywords for the wide range of SPME applications with regard to analysis of dried or otherwise processed plant raw material and even living plants. The next chapters will expand on SPME procedures focussing on different aspects with regard to screening, semi-quantitative analysis and quality control of especially terpenic volatiles from aromatic plants and products:

- *Applicability* of SPME vs. conventional distillation
- Analytical *reproducibility* and *analyte-response linearity*
- Studies of *chemotypes* and *cultivars*
- Terpene metabolism and *ontogenetic variation*
- *Quality control* of processed herbs

Most of the research data presented have not been published in neither scientific nor public media earlier and has to be seen as a result of extensive studies based on SPME applications at The Plant Biocentre in the past 5 years. Information about the respective extraction and analysis parameters are given in Chapter 7., *Appendices*, at the end of this paper.

4.1 SPME vs. Distillation: *Peppermint*, *Chamomile*, *Basil* and *Dill*

HS-SPME requires a minimum of laboratory equipment, in most cases no chemicals, and thus may compete with common EO isolation techniques such as hydrodistillation and steam distillation. As part of the biochemical studies at The Plant Biocentre, both techniques have been simultaneously used in EO volatile analyses in order to develop appropriate methods for different purposes. Additionally, HS-SPME has been introduced as a tool for routine laboratory analyses for strawberry flavour screening (Holt, 2001 and 2002; Rohloff et al., 2002a and 2002b). Although disadvantages such as analyte-selectivity, analyte-discrimination and sample-dependant reproducibility are factors that limit the applicability, SPME may be applied successfully to the screening and semi-quantitative analysis of terpenic volatiles as an alternative technique to distillation. As part of the national project "Norwegian Herb Production (NUP)" during the period from 1994-1998, preliminary tests have been carried out on selected essential oil drugs that offer marketing opportunities. Traditional herbs such as *Mentha x piperita* L., clones

'Hardanger' and 'Tøyen'), *Matricaria recutita* L., *Ocimum basilicum* L. and *Anethum graveolens* L. were analysed by HD sampling and HS-SPME.

Both techniques have been simultaneously applied in order to cover a broader range of analytical tools for terpene screening and identification of trace compounds in aromatic herbs (Rohloff, 1999; Rohloff et al., 2000a; Rohloff 2002a; Rohloff et al., 2002c). Eighty-six compounds were identified in rose root (*Rhodiola rosea* L.) based on EO analysis and HS-SPME (Rohloff, 2002b). Coleman and Lawrence (1997) carried out analyses of cedar wood EO comparing distillation, SHS-SPME, DHS on Tenax and SHS-gas-tight sampling. The characteristic main compounds α- and β-cedrene and thujopsene were detected by all methods with the exception of SHS sampling. The compound ratios were good reflected by both DHS and SPME, while the SPME method showed high affinity to α-pinene (32.4 %) compared to the EO samples (0.8 %). On the other hand, the RSD values from SPME sampling were relatively low (5.6 %) in contrast to DHS (10.5 %) and EO (3.1 %). Schäfer and co-workers (1995) studied needles of four *Pinus* species by applying both HS-SPME and SDE and reported a good estimation of monoterpene concentrations, but simultaneously pointed out the disadvantage of SPME when analysing multi-component mixtures with a wide range of boiling points and thus, distribution constants. Field and co-workers (1996) showed that HS-SPME might provide as good humulene/caryophyllene ratios in hops (*Humulus lupulus* L.) as those obtained by conventional SD and pentane extraction. Additionally, Kovačevič and Kač (2001) compared SD and HS-SPME methods for the determination and characterization of hop varieties by detecting the myrcene/humulene ratios (see also Section 4.3, *Identification of Chemotypes and Cultivars: Dill and Hops*). Paniandy and co-workers (2000) observed that more compounds could be identified in oil samples of guava fruits (*Psidium guajava* L.) compared to HS-SPME which is in accordance with the authors reports on rose root (Rohloff, 2002b). On the other hand, SPME revealed the occurrence of aldehydes, terpenes and phenylpropenes in guava that were not covered by SD analysis. It has to be mentioned that SPME offers an alternative method for determining EO volatiles when only very small sample amounts are available and SD and HD techniques are excluded. In this context, one should also consider reports of comparative analyses between SPME and other headspace techniques such as HSSE (Bicchi et al., 2000a), SHS (Miller and Stuart, 1999) and/or DHS applications (Matich et al., 1996; Vercammen et al., 2000). In Table 4-1, results from comparative studies of peppermint, chamomile, sweet balm and dill are summarized, presenting the character-impact terpenes and phenylpropenes. EO volatiles were isolated by conventional distillation and HS-SPME and analysed on a GC-MS system. In general, all compounds could be identified by both methods.

Table 4-1: Comparison of the EO volatile composition (%) from distillation (HD) and SPME sampling of peppermint, chamomile, sweet basil and dill (*t = trace compound)*.

PEPPERMINT	HD	SPME	CHAMOMILE	HD	SPME
1,8-cineole	4.7	20.3	**α-farnesene**	48.9	9.4
menthone	32.5	9.6	**spathulenol**	10.0	11.4
menthofuran	1.7	3.3	**bisabololoxide A**	2.3	30.1
isomenthone	3.0	3.0	**bisabolone oxide**	4.3	14.9
menthyl acetate	3.3	1.0	**chamazulene**	1.2	*t*
neomenthol	1.0	0.8	**bisabololoxide B**	15.2	8.5
menthol	44.5	16.7	**spiroether**	8.1	7.6
SWEET BASIL	**HD**	**SPME**	**DILL**	**HD**	**SPME**
1,8-cineole	9.6	31.7	**α-thujene**	*t*	0.5
linalool	17.7	38.8	**α-pinene**	1.5	3.6
estragole	34.0	12.0	**β-myrcene**	0.7	0.8
(*E*)-methyl cinnamate	5.8	3.2	**α-phellandrene**	39.5	34.0
methyl eugenol	10.5	*t*	***p*-cymene**	23.3	35.4
β-caryophyllene	4.4	1.4	**limonene**	19.3	20.8
τ-cadinol	6.0	0.2	**anethofurane**	5.0	4.5

Source: Rohloff, 1998 (unpublished data)

Peppermint oil is characterized by terpenes related to the *p*-menthane group of which menthol and menthone make up over 70 %, which is consistent with other reports (Voirin et al., 1990; Boelens, 1991; Marotti et al., 1994; Dragland and Aslaksen, 1997; Rohloff, 1999; Rohloff et al., 2000b; Wichtl, 2002). By using SPME, higher amounts of 1,8-cineole were obviously detected, and the menthol/menthone concentrations from the oil analysis were not reflected.

Chamomile oil is characterized by acyclic (farnesene) and monocyclic sesquiterpenes (bisabolol and its oxides, bisabolene) and spiroether (Iversen et al., 1994b; Zekovic et al, 1994; Das et al., 1998; Bicchi et al., 1999; Bottcher et al., 2001). In general, the SPME method performed well and detected all sesquiterpenes of interest, but the insufficient detection of the important compound chamazulene revealed the disadvantage of SPME.

Sweet Basil oil reveals high concentrations of phenylpropenes such as estragole, eugenol and methyl cinnamate as reported in many studies (Sheen et al., 1991; Grayer et al., 1996; Pino, 1999a; Lewinsohn et al., 2000; Ehlers et al., 2001; Gang et al., 2001; De La Pena et al., 2002; Diaz-Maroto et al., 2002a and 2002b). Incomplete and insufficient recovery of important EO volatiles were also observed here, in this case the chemical class of phenylpropene structures, of which methyl eugenol was only detected in trace amounts.

Dill herb oil is often used in food flavouring being described by many research groups (Halva, 1987; Halva et al., 1988; Boelens, 1991; Strunz et al., 1992; Pino et al., 1995; Pino, 1999b; Wust and Mosandl, 1999; Bailer et al., 2001). In the present study, the applied method reflected the "real" oil composition from HD sampling and underscored the applicability and usefulness of information one can gain when applying HS-SPME.

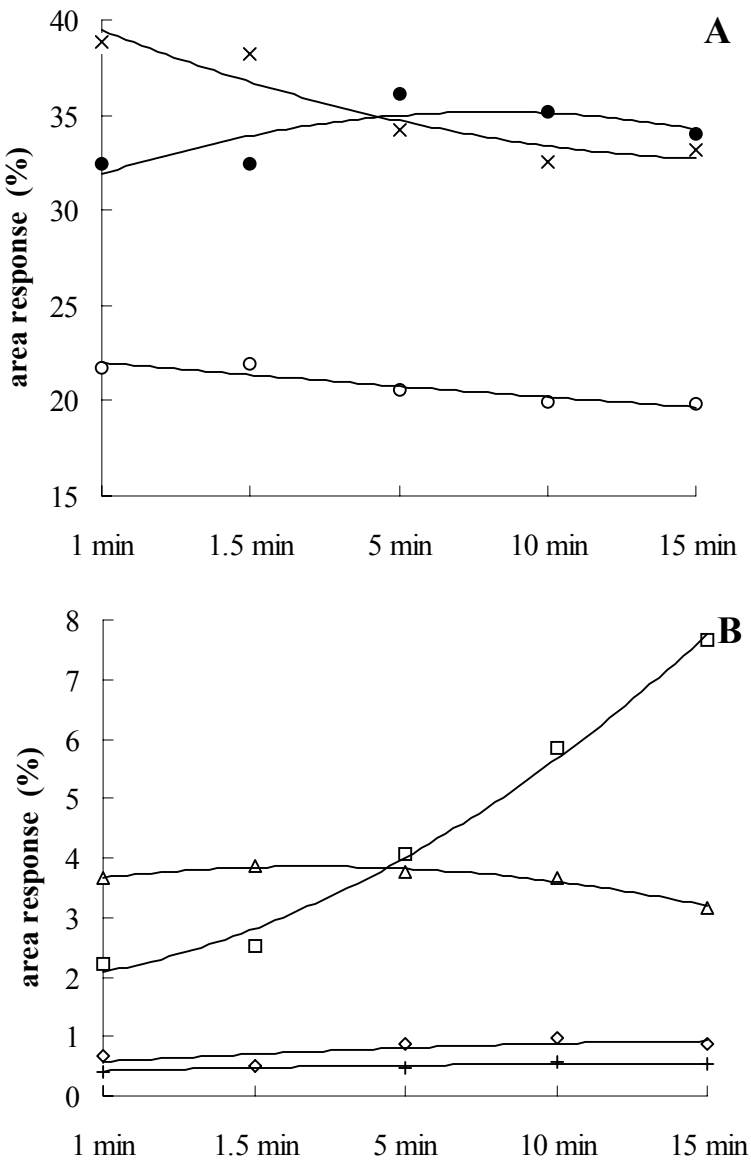
It has to be noted that the presented results were obtained by applying the same method for all samples without finding suitable extraction parameters that could match the results from oil analyses. This study underscores the problems one might have to deal with when using HS-SPME for the analysis of complex EO matrices with regard to varying boiling points, partition coefficients and affinity to the chosen fibre type.

4.2 Extraction Time and Concentration: *Dill* and *Sachalinmint*

Apart from the choice of fibre type, the use of suitable extraction conditions with regard to equilibrium time and temperature is crucial for successful HS-SPME applications. Many reports describe an equilibration procedure before the proper extraction by HS-SPME. Complete equilibration is attained when the partitioning of volatiles between the sample matrix and the headspace atmosphere stabilizes. When exposing the SPME fibre to the headspace, the analytes will then partition between the matrix, headspace *and* fibre coating and a new equilibrium must settle. Since only negligible amounts of analytes are on-fibre after exposure and the distribution of analytes in the sample matrix is almost unchanged (presuming the sample weight or volume is sufficient), the chemist might decide to drop sample equilibration and to begin directly with the extraction. In this case, one might meet the problem of discriminating effects, i.e. highly volatile compounds would partition easier into the gaseous phase and into the fibre. When using liquid absorption phases such as PDMS, one can wait until the equilibrium has been reached, whereas the use of solid coatings with high polarity and crystalline structure, e.g. CAR, might discriminate semivolatiles since adsorption sites on the fibre are already occupied. In one way, equilibrating before extraction is the best choice when dealing with HS-SPME.

DHS- and SHS-SPME applications using unequilibrated conditions (the socalled *breakthrough-mode*) have been widely reported and are useful for volatile screening purposes and qualitative analyses (e.g. Rohloff, 1999; Coleman and Lawrence, 2000; Coleman and Lawrence, 2002; Rohloff, 2002a; Zini et al., 2002). A temperature increase will lower equilibrium time, but on-fibre analyte concentrations will be decreased as a temperature effect (Nilsson et al., 1995; Jia et al., 1998). One could apply extremely short extraction times of only a few seconds or minutes (Coleman and Lawrence, 1997; Rohloff, 2002a) to obtain sufficient sensitivity. Lord and Pawliszyn (2000) point out that SPME in the breakthrough-mode is controlled by diffusion in contrast to partition-based extraction under equilibrated conditions. The accumulation of late eluting compounds is favoured by the latter as can seen in Figure 4-1 and 4-2 (*anethofuran*).

Figure 4-1: Effect of varying SPME exposure times on the fibre extraction efficiency with regard to characteristic terpenes from dill herb (*Anethum graveolens* L.).
A: • α-phellandrene, o limonene, × p-cymene. **B**: + α-thujene, Δ α-pinene, ◊ β-myrcene, □ anethofuran.



Source: Rohloff, 1997 (unpublished data)

For monoterpenes, an extraction time of about 10 min is often suitable, because analyte partitioning starts to stabilize, and in most cases, extraction times of 30 min are enough to reach equilibrium conditions. Ligor and Buszewski (1999) obtained the stabilization of menthol concentrations after 20-25 min.

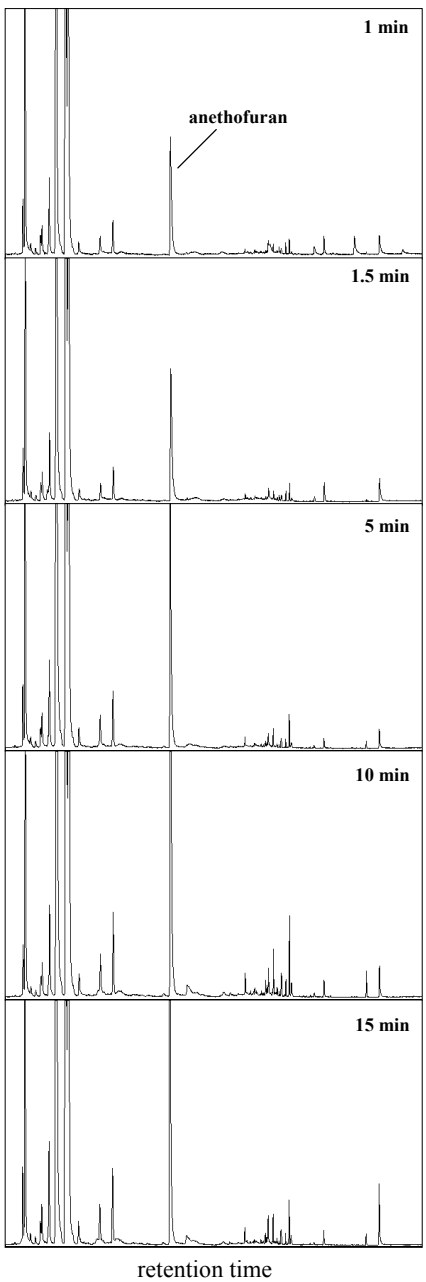


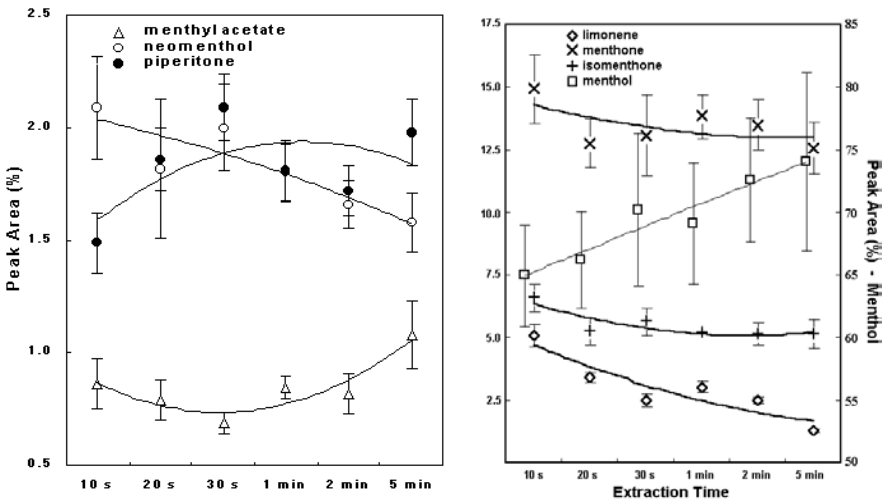
Figure 4-2: Effect of varying SPME exposure times (1, 1.5, 5, 10 and 15 min) on GC-MS detection (area %) of anethofuran from dill herb (*Anethum graveolens* L.).

Source: Rohloff, 1997 (unpublished data)

However, exceptions such as the continually increasing area response of the sesquiterpenes α- and β-caryophyllene from hops on a GC-FID system after 5 h exposure have been reported (Fields et al., 1996). Figures 4-1 and 4-2 illustrate the effect of different exposure times varying from 1 min up to 15 min, when analysing dill herb samples on a GC-MS system. The amounts of the character-impact compound *anethofuran* were still increasing after 15 min, whereas monoterpenes such as β-myrcene and *p*-cymene decreased. The ability to detect higher-boiling EO volatiles by increased fibre exposure times is clearly demonstrated in Figure 4-2.

Similar effects could also be observed when using SPME extraction times under 1 min (Figure 4-3). Sachalinmint EO is characterized by low complexity, with menthol as the dominating compound - up to or over 80 % - of the oil. The increase of fibre exposure time from 10 sec to 5 min resulted in still increasing amounts of menthol, while the fibre absorpstion of neomenthol and limonene continuously decreased. Of course, unequilibrated conditions will not reflect the real distribution of monoterpenes, as shown by the example of sachalinmint. One has to make a decision, whether it is suitable to operate with equilibrium phases or to operate in the breakthrough-mode, i.e. method development is inevitable when carrying out analyses of samples of unknown composition.

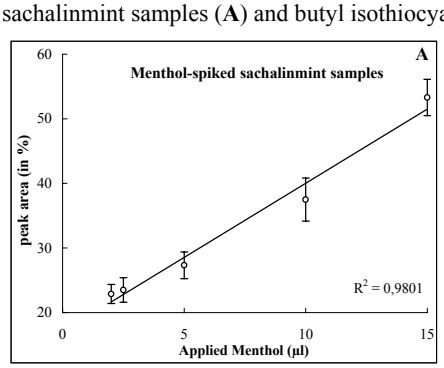
Figure 4-3: Headspace SPME sampling of sachalinmint samples (n=5) under unequilibrated conditions using extraction times between 10 s and 5 min.



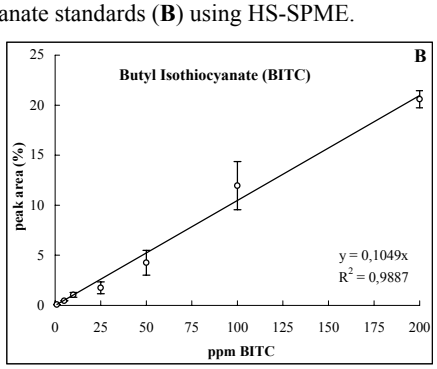
Source: Rohloff, 2002a (reprinted with permission from *J.Agric. Food Chem.* 50: 1543-1547, © 2002 American Chemical Society ACS)

Despite differing extraction time profiles, the HS-SPME procedure shows strict linearity over a wide concentration range concerning different chemical structures (Alpendurada, 2000) such as hydrocarbons, aldehydes, ketones, esters and alcohols (Jelen et al., 2000; Holt, 2001 and 2002), benzothiazoles (Bellavia et al., 2000), isothiocynates (Gandini and Riguzzi, 1997; Liu, 2000; Figure 4-4) and terpenes (Figure 4-4). Viewed from the background of partitioning effects, quantitative analyses of plant samples using HS-SPME seem to be rather complicated in contrast to environmental (Lloyd et al., 1998; Curren and King, 2001) or biomedical applications (Lord and Pawliszyn, 2000; Snow, 2000) when only one or a few analytes have to be traced. Consequently, equilibrium conditions should be preferred when quantifying complex sample matrices, and one has to take into account that the standard does not interfere with the sample when using the internal standard or standard-addition method.

Figure 4-4: Linearity of area response (%) vs. concentration for menthol-spiked sachalinmint samples (**A**) and butyl isothiocyanate standards (**B**) using HS-SPME.



Source: Rohloff, 2000 (unpublished data)

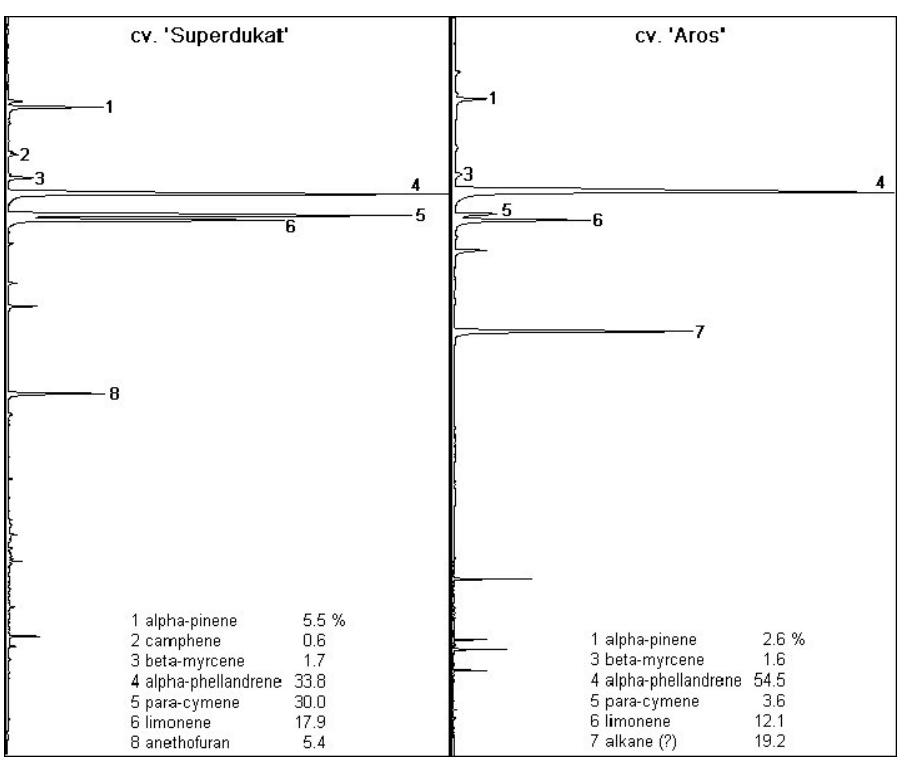


Source: Rohloff, 2001 (unpublished data)

4.3 Identification of Chemotypes and Cultivars: *Dill* and *Hops*

As already pointed out, SPME offers an excellent tool for fast and reliable qualitative analyses and shows high applicability for the screening of terpene volatiles from different sources with the aim of identifying chemotypes and cultivars. Applications for herbs and medicinal plants (Field et al., 1996; Krüger and Zeiger, 1996; Kovačevič and Kač, 2001; Rohloff et al., 2002c), flower aroma volatiles (Guidotti and Vitali, 1998; Verzera et al., 2000), fruits and berries (Ulrich et al., 1997; Beaulieu and Grimm, 2001; Holt, 2002), wine (Garcia et al., 1998a and 1998b; Vas et al., 1998; Pozo-Bayon et al., 2001;), coffee (Freitas et al., 2001) and tobacco (Clark and Bunch, 1998) have been described. In Figure 4-5 the example of GC-MS profiles of dill herb from two different cultivars, 'Superdukat' and 'Aros' is given. Monoterpenes such as α-pinene, β-myrcene, α-phellandrene, *p*-cymene and limonene occurred in both samples, whereas the characteristic aroma compound *anethofuran* (or dill ether) was only detected for 'Superdukat'. In fact, the lack or traces of anethofuran might be disadvantageous for flavouring properties, and the cultivars showed quite different amounts of important monoterpenes.

Figure 4-5: GC-MS chromatograms of SPME dill herb samples (*Anethum graveolens* L.) of two different cultivars – 'Superdukat' and 'Aros' – showing the variation of the main and characteristic terpene compounds.



Source: Rohloff, 1997 (unpublished data)

The analysis of hops was already mentioned in another context, since HS-SPME applications have been described for this species (Field et al., 1996; Kenny, 2000; Kovačevič and Kač, 2001). The commercial value of hops for the brewing of beer and the composition and flavour properties of hops EO volatiles have been studied in detail (Freundorfer et al., 1991; Green and Osborne, 1993; Auerbach et al., 2000; Eri et al., 2000; Kač and Kovačevič, 2000). Angelino (1991) pointed out the flavour potential of the "main" terpenes for beer production – α-caryophyllene (also named humulene) and β-caryophyllene. Since each variety has its own EO pattern, HS-SPME might be applied for the screening of chemical fingerprints and chemotypes, focussing on the ratios of humulene/ β-caryophyllene (*H/C ratio*) and the amounts of β-myrcene (Table 4-2).

Table 4-2: Chemotypic variation (in %) of HS-SPME-detected mono- and sesquiterpenes in hop cones (*Humulus lupulus* L.) collected from wild-growing plants in Norway.

Compound	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
α-phellandrene	0.4	0.3	0.5	0.5	0.5	0.5	0.7	0.6
β-myrcene	57.5	23.8	39.0	36.4	36.6	39.8	44.2	49.8
limonene	0.1	-	0.2	-	-	-	-	-
β-phellandrene	0.7	-	0.2	-	-	-	-	-
(*E*)-ocimene	0.4	0.3	1.0	1.3	1.5	-	0.6	0.4
α-copaene	0.4	0.6	-	0.6	0.5	0.7	0.5	0.5
1-octen-3-ol	-	0.3	-	0.2	0.5	-	-	0.5
α-cadinene	0.6	1.0	0.6	0.9	0.8	3.3	0.8	0.6
β-caryophyllene	11.7	20.1	12.0	16.5	9.4	12.5	12.1	15.8
2-undecanone	0.5	0.3	5.6	0.5	4.8	0.3	0.8	-
α- caryophyllene	19.0	34.5	21.1	31.0	2.6	20.0	23.0	24.4
γ-selinene	0.4	0.7	0.8	1.0	1.7	0.8	2.9	1.0
γ-muurolene	1.0	1.6	1.6	1.6	2.4	1.5	1.5	1.4
β-selinene	1.8	2.9	3.1	2.3	6.5	3.4	2.5	-
α-selinene	1.6	3.0	3.1	2.3	6.8	3.2	2.1	-
α-farnesene	-	0.2	-	-	-	-	-	1.6
δ-cadinene	1.5	2.8	1.2	2.3	1.8	1.6	2.1	2.0
Identified (%):	**97.6**	**92.4**	**90.0**	**97.4**	**76.4**	**87.6**	**93.8**	**98.6**

Source: Rohloff, 2001 (unpublished data; with permission of Steinar Dragland, Planteforsk)

Results from the terpene screening of wild-growing hops showed that the EO volatile distribution differed greatly. In sample no. 5 especially, a totally different fingerprint was observed in contrast to the other samples which were dominated by the already mentioned compounds of interest – β-myrcene, α- and β-caryophyllene, although none of the analysed hop samples reached an H/C ratio above 2.5 (the quality mark for female cones which are used in beer production). Simultaneously, the fingerprinting revealed the occurrence of other characteristic aroma volatiles, e.g. α-farnesene, which was only detected in samples no. 2 and 8 and which, in addition might contribute to the aroma when using hop for flavouring.

4.4 Ontogenetic Differentiation: *Peppermint*, *Sachalinmint* and *Arnica*

The distinct effect of ontogenetic development has already been discussed in detail in Chapter 2.4, "*Norwegian Herb Production (NUP)*" *1994-1998*, and will only be discussed here with relation to HS-SPME applications. As already described earlier, this technique can be used to screen the volatile composition of plant samples within short time intervals for the detection of biosynthetic and thus, qualitative changes (Beaulieu and Grimm, 2001; Maes et al., 2001). *Mentha* species such as peppermint and sachalinmint have been exhaustively studied at the Plant Biocentre in order to gain information about the variation of EO quality and composition with regard to growth stage and the portion of the different plant organs being harvested (Rohloff, 1999; Rohloff, 2002a). Lawrence (2002) pointed out the within-plant variation of EO volatiles, as shown for *Mentha pulegium* and *Melissa officinalis*, which might be of critical importance when taking plant samples for chemical analyses. This fact is illustrated in Tables 4-3 and 4-4, which present results from HS-SPME sampling of peppermint and sachalinmint when the leaves and flowers were collected from different stem positions. Evidently variations from basal leaves to top leaves and flowers can be observed, e.g. in the amounts of menthol and menthone. It has to be noted that highest concentrations of the undesirable menthofuran were detected in the flowers (Table 4-3).

Table 4-3: Characteristic terpene volatiles from flowers and leaves of peppermint (clone 'Hardanger') detected by HS-SPME (area response %).

Compound	Flowers	Leaves 1-3	Leaves 4-5	Leaves 6-7	Leaves 8-9
limonene	9.8	8.4	8.9	10.0	10.1
1,8-cineole	7.1	22.1	22.7	20.4	15.9
***p*-cymene**	1.9	2.6	2.9	3.1	2.6
(*E*)-*p*-menth-2-en-1-ol	0.8	2.3	2.3	3.1	3.2
menthone	38.1	32.9	14.4	10.3	6.7
menthofuran	12.6	2.6	4.4	3.7	2.7
isomenthone	3.4	3.7	2.9	3.0	2.4
menthyl acetate	0.3	0.6	0.7	1.0	1.6
neomenthol	1.4	1.8	1.9	2.7	3.2
β-caryophyllene	0.2	0.7	1.1	1.3	1.1
menthol	8.3	10.5	14.0	19.6	22.5
pulegone	0.9	-	0.3	0.2	0.2
piperitone	0.2	0.2	0.2	0.2	-

Source: Rohloff, 1999

Results from the EO volatile screening from sachalinmint showed similar tendencies regarding the distribution of menthol and menthone throughout the plant, whereas only trace amounts of menthofuran could be detected (Table 4-4).

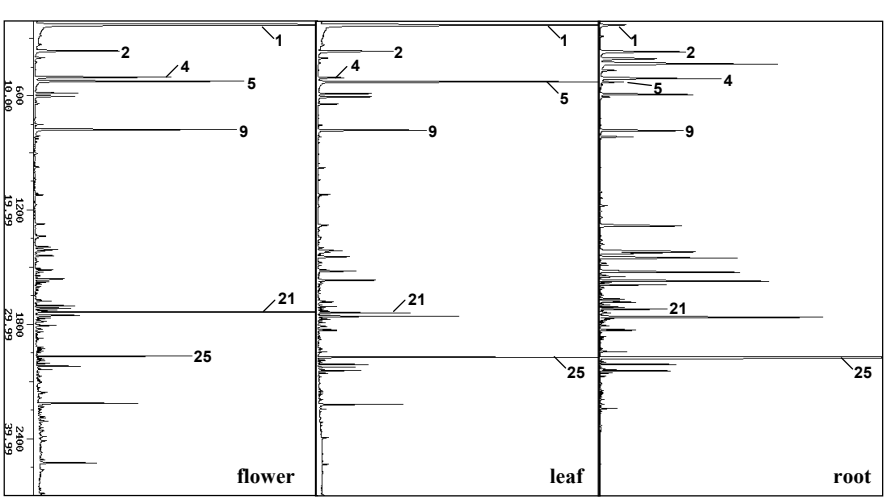
Table 4-4: Terpene composition (area response %) of sachalinmint leaves (and flowers) with regard to plant height: A – 0-20 cm; B – 20-40 cm; C – 40-60 cm; D – 60-80 cm.

Compound	A	B	C	D
menthol	**58.90** *±3.25*	**48.54** *±2.44*	**65.28** *±2.42*	**71.46** *±1.93*
menthone	**12.90** *±1.06*	**13.34** *±1.91*	**10.98** *±0.64*	**6.21** *±0.43*
isomenthone	**5.32** *±0.50*	**6.58** *±0.21*	**6.50** *±0.71*	**6.83** *±0.39*
menthyl acetate	**0.68** *±0.03*	**0.74** *±0.03*	**1.14** *±0.25*	**2.15** *±0.13*
neomenthol	**1.42** *±0.11*	**1.55** *±0.12*	**1.88** *±0.12*	**2.21** *±0.08*
piperitone	**1.31** *±0.10*	**1.43** *±0.35*	**1.68** *±0.04*	**1.89** *±0.12*

Source: Rohloff, 2002a (reprinted with permission from *J.Agric. Food Chem.*, 50: 1543-1547, © 2002 American Chemical Society ACS)

These results provide not only useful information for the correct sampling of plants or plant parts for EO analysis, but might be the starting point for further investigation of aromatic herbs with regard to more advanced harvesting techniques to obtain special EO qualities. Another example of varying EO composition is given in Figure 4-6 and Table 4-5, which present results from HS-SPME sampling of arnica (*Arnica montana* L.). Arnica is a famous medicinal plant that has been used for centuries for antiseptic, antiphlogistic and antirheumatic applications. Terpenes from arnica have been analysed by different methods (Güntzel et al., 1967; Rinn, 1970; Willuhn, 1972; Stausberg, 1994) and the occurrence of active metabolites within the chemical group of sesquiterpenelactones found in different plant organs has been studied (Leven and Willuhn, 1987; Leven, 1988; Willuhn and Leven, 1991; Stausberg, 1994).

Figure 4-6: GC-MS example chromatograms from dried flower, leaf and root samples of arnica (*Arnica montana* L.) detected by HS-SPME. For numbering of selected compounds, see the following table (Table 4-5).



Source: Rohloff, 1999 (unpublished data)

Table 4-5: Distribution of terpene volatiles and thymol derivates from dried flowers, leaves and roots of arnica (*Arnica montana* L.) detected by HS-SPME.

No.	Compound	Flowers	Leaves	Roots
1	**α-pinene**	25.3	29.1	1.0
2	**β-pinene**	4.1	4.4	4.4
3	**sabinene**	0.5	0.5	2.5
4	**α-phellandrene**	6.3	1.3	7.0
5	**β-myrcene**	9.5	14.3	0.6
6	**α-terpinene**	-	-	7.5
7	**limonene**	2.0	2.2	3.7
8	**β-phellandrene**	1.8	2.2	0.2
9	***p*-cymene**	8.7	4.2	3.5
10	**terpinolene**	-	-	0.9
11	**6-methyl-5-hepten-2-one**	0.4	0.3	-
12	**(*E*)-2-hexen-1-ol**	0.4	0.4	-
13	**nonanal**	0.7	-	-
14	**acetic acid**	0.5	-	-
15	**decanal**	1.5	-	-
16	**bornyl acetate**	-	-	0.4
17	**β-caryophyllene**	1.5	2.7	5.5
18	**thymol methyl ether**	0.2		1.6
19	**α-caryophyllene**	0.4	0.6	0.8
20	**(*E*)-β-farnesene**	-	0.7	0.4
21	**germacrene D**	8.1	5.2	2.0
22	**dodecanal**	1.3	3.9	-
23	**germacrene B**	0.3	-	-
24	**α-farnesene**	0.5	0.7	0.3
25	**thymol isobutyrate**	1.4	11.2	20.0
26	**tetradecanal**	0.3	0.2	-
27	**8,9-didehydro thymol isobutyrate**	0.3	1.1	1.6
28	**(*E*)-nerolidol**	0.3	-	-
29	**hexadecanal**	0.2	-	-
30	**thymol**	0.3	1.1	1.5

Source: Rohloff, 1999 (unpublished data)

In Table 4-5, a list of 30 different monoterpenes and sesquiterpenes, phenols and aldehydes, identified in the flowers, leaves and roots of arnica is presented. The highest amounts of species-characteristic terpenoid phenols (*thymol* and derivates) were detected in the roots, whereas the aerial parts of arnica were characterized by higher portions of monoterpenes (α-pinene, β-myrcene and *p*-cymene) and the sesquiterpene germacrene D. Again, the importance of knowledge of the within-plant variation of terpenes and other volatile compounds is illustrated and underscored the usefulness for optimised harvesting techniques and medicinal applications.

4.5 Quality Control of Processed Herbs: *Basil* and *Coneflower*

Drying of plant products is one of the oldest techniques for conservation of the inner quality, i.e. important primary and secondary products of food for short- and long-term storage. The use of microwave application for preservation, pasteurisation and sterilization by short-term thermal treatments is a relatively new method, which has also been applied to spices and herbs (Farag et al., 1996; Farag and El-Khawas, 1998). Possible negative effects of other common methods such as irradiation (Fan and Gates, 2001; Koseki et al., 2002) and UV radiation (Lamikanra et al., 2002) on terpene volatiles have been discussed.

Table 4-6: Variation in monoterpene, sesquiterpene and phenylpropene composition (area response %) of dried samples (40°C) of sweet basil (*Ocimum basilicum* L.) after microwave treatment detected by HS-SPME (*t* = *trace compound*).

Compound	Control	Microwave 15 sec	Microwave 30 sec
α-pinene	1.5	0.4	0.2
sabinene	0.2	*t*	*t*
β-pinene	1.0	0.9	1.0
β-myrcene	3.7	*t*	*t*
1,8-cineole	30.2	3.9	2.9
linalool	38.5	25.3	24.3
camphor	0.8	0.5	*t*
estragole	11.3	19.1	12.2
(*Z*)-methyl cinnamate	*t*	0.2	0.2
α-copaene	0.3	1.3	0.9
eugenol	0.1	0.8	0.3
(*E*)-methyl cinnamate	3.4	10.2	15.0
methyl eugenol	0.3	4.2	1.8
β-caryophyllene	1.3	5.0	5.0
(*Z*,*E*)-α-bergamotene	3.4	9.9	14.1
α- caryophyllene	0.3	1.3	1.2
(*Z*)-β-farnesene	0.1	0.7	0.4
germacrene D isomer	0.1	0.6	0.6
germacrene D	0.7	2.6	2.5
β-selinene	*t*	0.6	0.6
valencene	0.3	1.0	1.3
viridiflorene	0.3	1.1	1.7
β-bisabolene	*t*	0.4	0.4
γ-cadinene	0.7	2.7	4.4
(*Z*)-calamene	0.1	0.4	0.7
(*E*)-β-farnesene	0.1	0.3	0.4
(*Z*)-γ-bisabolene	0.1	0.9	0.4
cubenol	*t*	0.3	0.5
τ-cadinol	0.2	1.1	2.0

Source: Rohloff, 1997 (unpublished data)

Many HS-SPME applications have already been reported dealing with the screening and monitoring of changes of the composition of flavour and volatile compounds due to thermal treatment of food (Servili et al., 2000), pharmaceutical products (Besse et al., 2001; Coran et al., 2001; Karaisz and Snow, 2001) and spices (Roberts and Pollien, 1997). Flavour loss as an effect of microwave treatment has been reported by Roberts and Pollien (1997) and the influence of irradiation (Koseki et al., 2002) and microwave (Antonelli et al., 1998; Silva et al., 1999; Yousif et al., 1999; Di Cesare et al., 2001) on EO volatiles of sweet basil (*Ocimum basilicum* L.) has been observed. In Table 4-6 an example of the effects of short-term thermal treatment of pre-dried basil samples on monoterpene, sesquiterpene and phenylpropene composition is given. The amount of SPME-extracted compounds shifted from monoterpenes volatiles to less volatile sesquiterpenes and phenylpropenes, and the portion of 1,8-cineole was distinctly decreased. It has to be noted that the detected amounts of monoterpenes in the control group might be too high (due to short SPME exposure time of 1 min), and the area response of character-impact compounds with phenylpropene structure (estragole, methyl cinnamate, methyl eugenol) was not properly reflected by HS-SPME. However, it was shown that short-term microwaving clearly led to dramatic changes of basil aroma volatiles, which might be disadvantageous for the flavour properties of this herb.

Another method for the conservation and processing of medicinal plants is the EtOH-extraction of active metabolites. One of the most commonly used plant immunostimulants nowadays is *Echinacea*, or coneflower, used for the treatment of colds and flu. Three different species are of medicinal interest due to their content of effective alkylamides and polysaccharides: *E. purpurea* (L.) Moench, *E. angustifolia* DC. and *E. pallida* Nutt. The occurrence of their active compounds and biochemistry has been reported (Bauer et al., 1988a and 1988b; Bauer and Remiger, 1989; Bauer and Wagner, 1990) and their EO composition has been described (Bauer and Wagner, 1990; Schulthess et al., 1991; Wagner, 1995; Mazza and Cottrell, 1999; Millard et al., 1999). As part of a quality screening study, eight commercially accessible herbal remedies and preparations mainly produced from red coneflower (*E. purpurea*) and sold on the Norwegian market, have been studied by HS-SPME analyses.

Table 4-7: Description of the tested herbal remedies of *E. purpurea* preparations, *E. angustifolia* (no. 7) and a mix of *E. purpurea*, *angustifolia* and *pallida* extracts (no.8).

Sample	**no. 1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Plant organ	*Herba Flos Radix*	*Herba Flos Radix*	*Radix*	*Herba Flos Radix*	*Herba*	*Herba*	*Herba*	*Radix*
Base	60 % EtOH	60 % EtOH	33 % EtOH	60 % EtOH	60 % EtOH	Tablet	50 % EtOH	80:20 Glycerol :H_2O

Source: Rohloff, 1999 (with permission by Biosan Pharma, Trondheim; unpublished data)

Table 4-8: Screening of the terpene volatile composition of eight commercially available herbal remedies from *Echinacea* extracts detected by HS-SPME (*t= trace compound*).

MONOTERPENES								
Compound	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
β-pinene	0.37	*t*		1.06	*t*	0.49	1.29	
sabinene	*t*					0.50		
α-phellandrene	0.65	*t*		0.54	*t*	*t*	*t*	
β-myrcene	3.61	2.26	*t*	*t*	0.50			
limonene	0.37	t	*t*	*t*	2.30	8.58	*t*	*t*
1.8-cineole			*t*		*t*	0.16	*t*	0.66
β-phellandrene	*t*	*t*		*t*	*t*		*t*	
γ-terpinene	*t*	*t*	*t*	*t*	*t*	1.45	*t*	*t*
(*E*)-ocimene	*t*	*t*		*t*		*t*		
***p*-cymene**	0.73	*t*	*t*	0.77	0.98	0.95	*t*	*t*
terpinolene			*t*			0.49	*t*	
Total area (%)	**5.73**	**2.26**	**-**	**2.37**	**3.78**	**12.26**	**1.29**	**0.66**
TERPENE ALCOHOLS and PHENYLPROPENES								
4-terpineol								0.23
neomenthol								1.72
estragole			*t*		0.32		1.25	
α-terpineol								1.33
(*E*)-anethole			9.00	0.82	4.73		34.90	0.19
(*E*)-nerolidol	*t*	*t*	*t*		*t*		*t*	
eugenol						52.40		
thymol						*t*	*t*	
Total area (%)	**-**	**-**	**9.00**	**0.82**	**5.05**	**52.40**	**36.15**	**1.75**
TERPENE ESTERS								
linalyl acetate								3.39
bornyl acetate						*t*		0.16
nerolidyl acetate								*t*
Total area (%)	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**3.55**
SESQUITERPENES								
α-copaene	1.06	1.40	2.02	1.96		0.54	2.77	
β-bourbonene	*t*	*t*		*t*	0.36		*t*	
β-cubebene	1.19	1.28		0.85				
β-caryophyllene	6.27	4.52		9.67	10.96	4.25	5.67	11.89
α-caryophyllene				*t*			0.68	*t*
α-guaiene	1.68			2.48	2.36			
germacrene D	22.51	67.48		39.65	29.60	0.58	3.98	*t*
caryophyllene oxide		*t*		*t*	*t*	0.21	*t*	
Total area (%)	**32.71**	**74.68**	**2.02**	**54.61**	**43.28**	**5.58**	**13.10**	**11.89**

Source: Rohloff, 1999 (with permission by Biosan Pharma, Trondheim; unpublished data)

Due to commercial interests related to the results of this study, none of the tested remedies will be mentioned by name or trademark. Six ethanol-based *Echinacea* extracts, one tablet-based and one glycerol-based preparation were included in the test (Table 4-7). The aim of the study was to screen the samples for organic volatiles (terpenic compounds and phenylpropenes) and to get information about the qualitative composition

of the respective *Echinacea* preparations. The test results are presented in Table 4-8 and divide the detected compounds into five chemical groups: monoterpenes, terpene alcohols and phenylpropenes, terpene esters and sesquiterpenes.

Regarding the class of monoterpenes, most of the known compounds reported earlier could be identified in all preparations being processed from aerial plant parts, in contrast to sample no. 8, which was produced from root material. The amount of detected monoterpenes was especially good in samples no. 1 and no. 6, whereas only traces were detected in sample no. 3. When looking at the identified terpene alcohols and phenylpropenes, it becomes clear that the high amounts of (*E*)-anethole in sample nos. 3, 5 and 7 are not derived from *Echinacea* plant material, i.e. flavours (e.g. EO from anise) have been added to improve the taste of the phytopharmaceutical preparations. Extremely high contents of the phenylpropene eugenol in sample no. 6 revealed the occurrence of EO from another plant species, which is supposed to be clove. In which way this addition might influence the detection of monoterpenes and thus, the distribution of monoterpenes from *Echinacea* raw material, cannot be answered without additional product information. In the sesquiterpene group, high amounts of germacrene D, up to over 50 %, were detected in sample nos. 1, 2, 4 and 5, which is in accordance with reports by other authors who observed high germacrene contents in flower parts of *Echinacea* species. In addition, the naturally occurring sesquiterpene in coneflower, β-caryophyllene, was detected in sufficient amounts in the range of 5-10 % in all samples (except no. 3).

When summarizing all results, sample no. 3 showed the lowest amounts of terpenic compounds. This does not necessarily mean that the quality of this preparation was poor, since this study only focussed on EO volatiles and not on the immunostimulatory active metabolites (the EtOH content of this extract was much lower compared to the other ethanolic preparations). As already mentioned, high amounts of added EO flavours in sample no. 6 resulted in a distortion of the correct volatile composition. On the other hand, this preparation, together with sample nos. 1, 2, 4 and 5, covered a broad range of naturally occurring terpene volatiles and might be one hint for describing the fine quality of these preparations with regard to careful extraction and processing procedures.

5. Prospects of Aromatic Herbs ✧ A SCENT OF THE 21st CENTURY

When choosing the resolutions of the "Second World Congress on Medicinal and Aromatic Plants for Human Welfare WOCMAP-II" (Giberti et al., 1999) as the starting point for a discussion about the prospects of aromatic herbs, it became quite clear that their production, processing and application has been attracting increasing interest. The growing demand for medicinal and aromatic plants (MAPs) implies economic importance within the fields of agriculture, medicine, pharmaceutical industry and biotechnology, and makes the sustainable use of nature and its plant resources imperative, as well as the extension of quality control measures and statutory regulations for production and marketing. The common ground on which the international co-operation is based is highlighted by the WOCMAP-II postulations about concerted efforts for:

- *Taxonomic research on the native flora and search for novel compounds*
- *Establishment of pharmacological and toxicological information systems*
- *Standardization and regulation of phytotherapeutic preparations*
- *Application of Good Manufacturing Practice (GMP) in industrial processes*
- *Recording and protection of ancestral knowledge related to traditional phytomedicine*
- *Elaboration of guidelines for domestication and cultivation methods of MAPs*

These efforts seem to manifest themselves when reading the research topics from more recent international conferences on MAPs (Bernáth et al., 2002; Figueiredo et al., 2002; Ming et al., 2002). In the near future, novel plant volatiles will be discovered and new fields of EO utilization will be introduced. The chemical group of natural terpenoids especially seems to possess a promising potential for future applications. Since these compounds act as signalling messengers in most organisms and are involved in the interaction between biological systems that are quite diverse, scientists from several disciplines focus their work on bioactive terpenoids, thus leading to intensified interdisciplinary research. Molecular biology in particular should reveal additional biosynthetic pathways and the structure and function of corresponding enzymes, and extend biotechnological approaches and the industrial-scale production of plant secondary metabolites.

Discoveries and implementations within aromatic plant production, research and industrial processing go hand-in-hand with the development of newer instruments and analytical techniques. Adequate extraction, detection and structure elucidation are the key words within natural product chemistry and EO analysis. A great deal has changed since the publication of Sandra and Bicchi's comprehensive book, "*Capillary Gas Chromatography in Essential Oil Analysis*" (1987), and modern extraction and chromatographic techniques for the study of EO volatiles have been recently reported and reviewed (Ammann et al., 1999; De Castro et al., 1999; Adams, 2001; Gomez and Witte, 2001; Lang and Wai, 2001; Lockwood, 2001; Marriott et al., 2001; Hyötyläinen and Riekkola, 2002; Kubeczka, 2002; Sandra et al., 2002). Regarding the conclusions of Maarse

(1991) and Boelens (1991), many of their predictions about future trends within analytical instrumentation and research have come true during the past ten years. Smaller instruments, hyphenated techniques and automation processes have been implemented. The breakthrough of computer technology and database networking via the Internet especially has made the establishment of computer-based *expert systems* for end-users feasible. Despite technological innovations in the chromatographic sciences, sample preparation and extraction of plant volatiles is still the crucial step with regard to accuracy, precision and analysis time as pointed out by Sandra and co-authors (2002).

Modern biotechnology with PCR and micro-array technique provide excellent tools to study the biosynthesis of secondary metabolites on the molecular level. Laboratory work and instrumentation on a microscale for the extraction, detection and identification of plant volatiles will be further improved and established, and recently introduced techniques such as micro-SPE, single-drop extraction and SPME indicate the direction of miniaturization. Fast sample throughput, minimal sample size, organic solvent consumption and high sensitivity are of essential importance for such future developments. The application of dynamic sampling, on-site analysis and "lab-on-a-chip-technologies" has the potential to support real-time decisions, interactive sampling and cost-effective solutions in industrial processes.

The extent to which SPME might be implemented in automated quality control and chemical and manufacturing processes depends on developments and modifications of the (normally) manually operated SPME holder. Solid-phase microextraction seems to be an analytical tool for research applications rather than a generally applicable extraction technique. Its advantages over classical distillation and headspace applications are impressive due to drastically reduced analysis time and will introduce new frontiers in plant volatile research. As a newly reported, excellent example, Grassi and co-workers (2002) have literally used solid-phase "microextraction" to extract the EO liquids from single peltate trichomes! SPME needs further development with regard to quantification. Fibres with affinity coatings for target analytes and chiral coatings for optically active analytes will be developed, and the number of applications based on instrument hyphenation will increase. The user-friendliness of operating SPME will initiate the development of applications and "handy" equipment for the monitoring of volatiles for biological and environmental studies, extraction automation, on-site sampling and on-fibre storage of analytes. — "When talking about the direction in which future technology and research might grow, means always: Pointing at your Feet."

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7. APPENDICES - *Materials and Methods*

Chapter 4.1 — SPME vs. Distillation: *Peppermint*, *Chamomile*, *Basil* and *Dill*

Chamomile (*Matricaria recutita* L.), sweet basil (*Ocimum basilicum* L.) and dill (*Anethum graveolens* L.) were grown from seeds while peppermint plants (*Mentha x piperita* clone 'Hardanger') were grown by runner propagation. All species were pre-cultivated in the greenhouse at 25ºC at The Plant Biocentre over an 8-week period. In the end of June, all species were transferred to the trial field by planting them in 3 m long double-rows on 20 cm high, plastic-covered beds with 20 cm of space between plants and 25 cm between double-rows. Leaves and flowers of blooming peppermint and flowers from chamomile were harvested in early September, while leaves and stems of sweet basil and dill were harvested at the end of August 1997. All samples were dried at 35°C for 48 h and stored at room temperature prior to analysis in February 1998. The essential oil (EO) was obtained by separating leaves and/or flowers from the stems and crushing before distillation (Rohloff, 1999). GC samples were prepared by diluting 25 µl of essential oil in 1 ml EtOH and storing at 4°C prior to analysis. The SPME procedure was carried out by using a PDMS-coated fibre (100µm) as described by Rohloff (1999). All samples were analysed using a Varian Star 3400 CX gas chromatograph coupled with a Saturn 3 mass spectrometer with He as the carrier gas. *EO GC-MS conditions* – capillary column DB-5, 30 m, 0.25 mm i.d., 0.25 µm film, 35-200°C at 2.9°C min^{-1}, injector 250°C (split 1 min), detector 200°C (EI mode). *SPME-GC-MS conditions* – column as above, 35°C for 2 min, 35-250°C at 5°C min^{-1}, injector (split 2 min) and detector temperature as above.

Chapter 4.2 — Extraction Time and Concentration: *Dill* and *Sachalinmint*

Dill
For details on cultivation and sample preparation, see Chapter 4.1. *SPME conditions* – 1 g of dill herb (*Anethum graveolens* L. var. 'Superdukat') was sealed in a 10 ml screw-top vial with PTFE/silicone septum, equilibrium time 10 min at 35°C, extraction 10 min, desorption 2 min. *SPME-GC-MS conditions* – capillary column DB-5, 30 m, 0.25 mm i.d., 0.25 µm film, 35°C for 2 min, 35-250°C at 5°C min^{-1}, injector 220°C (split 2 min), detector 200°C (EI mode).

Sachalinmint
Sachalinmint plants (*Mentha sachalinensis* Briq. (Kudô) var. 'Mentolcsepp') were harvested at the Plant Biocentre, Trondheim, in September 1998 and dried at 35°C for 48 h. Samples (3 replicates) taken from a pooled leaf sample were spiked with menthol: 2, 2.5, 5, 10 and 15 µl. *SPME conditions* – 1 g plant sample in 10 ml screw-top vial with phenolic

cap and PTFE/silicone septum, equilibrium time 10 min at 45°C, extraction 30 s, desorption 3 min. *SPME-GC-MS conditions* – capillary column CP Wax 52CB, 30 m × 0.32 i.d., 0.25 µm film, 35ºC for 2 min, 35-250ºC at 5ºC min^{-1}, hold 2 min, injector 250ºC (split 2 min), detector 175ºC (EI mode).

Butyl Isothiocyanate (BITC)
BITC standard solutions (1, 5, 10, 25, 50, 100 and 200 ppm in H_20:EtOH 50:50) were prepared in 10 ml screw-top vials with phenolic cap and PTFE/silicone. *SPME conditions* – equilibrium time 10 min at 23°C for 24 h, extraction 10 min, desorption 2 min. *SPME-GC-MS conditions* – capillary column CP Wax 52CB, 30 m × 0.32 i.d., 0.25 µm film, 35ºC for 2 min, 35-250ºC at 5ºC min^{-1}, hold 2 min, injector 250ºC (split 2 min), detector 175ºC (EI mode).

Chapter 4.3 — Identification of Chemotypes and Cultivars: *Dill* and *Hops*

Dill
Dill leaves (*Anethum graveolens* L.) of two cultivars – 'Superdukat' and 'Aros' – were harvested in 1997 from non-flowering plants grown at Hordaland County, Norway, and oven-dried at 40°C. SPME analysis with a PDMS fibre (100 µm) was carried out in the same way as described by Rohloff (1999). All samples were analysed using a Varian Star 3400 CX gas chromatograph coupled with a Saturn 3 mass spectrometer with He as the carrier gas. *SPME conditions* – equilibrium time 30 min at 40°C, extraction 10 min, desorption 2 min. *SPME-GC-MS conditions* – capillary column DB-5, 30 m, 0.25 mm i.d., 0.25 µm film, 35°C for 1 min, 35-90°C at 2°C min^{-1}, 90-250°C at 15°C min^{-1}, injector 250°C (split 2 min), detector 200°C (EI mode).

Hops
The plant material was collected from wild-growing Norwegian plants and cultivated at the Research Station Kise, The Norwegian Crop Research Institute (Planteforsk) in Hedmark County. Hops cones (*Humulus lupulus* L.) of 8 different clones were harvested at the mature stage in October 2000 and immediately stored at -20°C prior to analysis in March 2001. All samples were analysed using a Varian Star 3400 CX gas chromatograph coupled with a Saturn 3 mass spectrometer with He as the carrier gas. *SPME conditions* – Each sample (20 g, frozen) was sealed in an Erlenmeyer flask (250 ml), equilibrium time 20 min at 50°C, extraction time 10 min (100 µm PDMS fibre), desorption 2 min. *SPME-GC-MS conditions* – capillary column CP Wax 52CB, 30 m × 0.32 i.d., 0.25 µm film, 35ºC for 2 min, 35-250ºC at 5ºC min^{-1}, hold 2 min, injector 250ºC (split 2 min), detector 175ºC (EI mode).

Chapter 4.4 — Ontogenetic Differentiation: *Peppermint*, *Sachalinmint* and *Arnica*

Pepprmint
Both leaves and flowers of *Mentha* × *piperita* (clone 'Hardanger') were harvested from trial plots at The Plant Biocentre, Trondheim. The sampling was carried out with regard to leaf position in basipetal direction by dividing the samples into the following groups: flowers, leaves 1-3, leaves 4-5, leaves 6-7, leaves 8-9. Samples were dried at 35°C for 48 h. Sample preparation, SPME (100 µm PDMS fibre) and GC-MS conditions are described in detail by Rohloff (1999).

Sachalinmint
Sachalinmint plants (*Mentha sachalinensis* Briq. (Kudô) var. 'Mentolcsepp') where harvested at the Plant Biocentre, Trondheim, in September 1998 and dried at 35°C for 48 h. The samples (3 replicates) were divided into 4 groups with regard to leaf (and flower) position: A – 0-20 cm; B – 20-40 cm; C – 40-60 cm; D – 60-80 cm. *SPME conditions* – 1 g plant sample in 10 ml screw-top vial with phenolic cap and PTFE/silicone septum, equilibrium time 10 min at 45°C, extraction 30 s, desorption 3 min. Plant material used for exposure time testing (10 s, 20 s, 30 s, 1 min, 2 min, 5 min) was taken from group D and equilibrated for 10 min at 45°C. *SPME-GC-MS conditions* – capillary column CP Wax

52CB, 30 m × 0.32 i.d., 0.25 µm film, 35ºC for 2 min, 35-250ºC at 5ºC min^{-1}, hold 2 min, injector 250ºC (split 2 min), detector 175ºC (EI mode).

Arnica

The dried plant material of *Arnica montana* L. harvested in 1999, was obtained from a farmer at Spideberg, Østfold County, in Southeastern Norway. Flower, leaf and root samples were analysed by using a 100 µm PDMS fibre. *SPME conditions* – 1 g plant sample in 10 ml screw-top vial with phenolic cap and PTFE/silicone septum, equilibrium time 15 min at 50°C, extraction 1 min, desorption 2 min. *SPME-GC-MS conditions* – capillary column CP Wax 52CB, 30 m × 0.32 i.d., 0.25 µm film, 35ºC for 2 min, 35-250ºC at 5ºC min^{-1}, hold 2 min, injector 250ºC (split 2 min), detector 175ºC (EI mode).

Chapter 4.5 — Quality Control of Processed Herbs: *Basil* and *Coneflower*

Sweet Basil

The SPME procedure was carried out on commercially available dried herbs (*Ocimum basilicum* L.) by using a PDMS-coated fibre (100µm) as described by Rohloff (1999). All samples were analysed using a Varian Star 3400 CX gas chromatograph coupled with a Saturn 3 mass spectrometer with He as the carrier gas. *SPME-GC-MS conditions* – column DB-5, 30 m, 0.25 mm i.d., 0.25 µm film, 35°C for 2 min, 35-250°C at 5°C min^{-1}, injector 250°C (split 2 min), detector 200°C (EI mode).

Coneflower sp.

Commercially available phytomedicinal remedies of *Echinacea purpurea* L. (nos. 1-6), *Echinacea angustifolia* L. (no. 7) and a preparation containing a root extract of *E. purpurea*, *E. angustifolia* and *E. pallida* (no. 8) were analysed by HS-SPME with regard to the composition of terpene volatiles. All preparations were EtOH-based (except for nos. 6 and 8) and analysed without further preparation. Sample no. 6 consisted of tablets of which 1 tablet was dissolved in 5 ml 60 % EtOH. Sample no. 8 consisted of a glycerol:H_2O (80:20) base and was analysed as is (5 ml). *SPME conditions* – 5 ml of each sample were sealed in a 10 ml screw-top vial with PTFE/silicone septum, equilibrium time 60 min at 50°C, extraction 60 min, desorption 2 min. *SPME-GC-MS conditions* – capillary column CP Wax 52CB, 30 m × 0.32 i.d., 0.25 µm film, 35ºC for 5 min, 35-90ºC at 3ºC min^{-1}, 90-210ºC at 7ºC min^{-1}, hold 15 min, injector 240ºC (split 2 min), detector 175ºC (EI mode).

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Volatiles from rhizomes of *Rhodiola rosea* L.

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Abstract

Terpenes and aroma volatiles from rhizomes of *Rhodiola rosea* L. from Norway have been isolated by both steam distillation and headspace solid-phase micro-extraction coupled with gas chromatography and mass spectrometry analysis. The dried rhizomes contained 0.05% essential oil with the main chemical classes: monoterpene hydrocarbons (25.40%), monoterpene alcohols (23.61%) and straight chain aliphatic alcohols (37.54%). *n*-Decanol (30.38%), geraniol (12.49%) and 1,4-*p*-menthadien-7-ol (5.10%) were the most abundant volatiles detected in the essential oil, and a total of 86 compounds were identified in both the SD and HS-SPME samples. Geraniol was identified as the most important rose-like odour compound besides geranyl formate, geranyl acetate, benzyl alcohol and phenylethyl alcohol. Floral notes such as linalool and its oxides, nonanal, decanal, nerol and cinnamyl alcohol highlight the flowery scent of rose root rhizomes.

Keywords: *Rhodiola rosea*; Crassulaceae; GC–MS; Headspace SPME; Terpenes; Aroma volatiles

1. Introduction

Rhodiola rosea L. or rose root, also commonly known as golden root and arctic root, is a perennial herbaceous plant of the family Crassulaceae. In all, 14 homonyms and infraspecific taxa have been described for *Rhodiola rosea*. The yellow-flowered taxon of rose root is spread widely and can be found in the mountain regions of Central and Northern Europe as well as in Russia and in the east coastal regions of North America at altitudes between 1000 and 5000 m above sea level. Rose root has an approximate plant height of 75 cm, with the characteristic flower scent of roses which impart its name.

Rose root is a multipurpose medicinal plant with adaptogenic properties by increasing the body's non-specific resistance and normalizing functions, and it has traditionally been grown and used in Russia and Mongolia for the treatment of long-term illness and weakness due to infection. Due to changing consumer demands towards natural health products and the growing interest for unknown plant secondary metabolites and their applications in biotechnology and therapy in the last decades, a great deal of focus has been put on rose root and its medical properties with regard to memory and learning (Petkov et al., 1986), immune response (Lishmanov et al., 1987; Furmanowa et al., 1999b), organ function, CNS and stress (Sokolov et al., 1985; Maslova et al., 1994; Afanas'ev et al., 1996; Lishmanov et al., 1999) and cancer therapy (Bocharova et al., 1994, 1995; Udintsev and Shakhov, 1991; Razina et al., 2000).

The chemical composition of rhizomes of *Rhodiola rosea* has been exhaustively studied by East European research groups (Khnykina and Zotova, 1966; Saratikov et al., 1967; Dubichev et al., 1991; Revina et al., 1976; Komar et al., 1980; Kurkin et al., 1986; Furmanowa et al., 1999b). Active metabolites within the chemical groups of phenols such as salidroside and its aglycon tyrosol (Peshekova et al., 1973; Kurkin et al., 1989; Kur'yanov et al., 1991; Satsyperova et al., 1993; Antipenko and Kuznetsov, 1998; Linh et al., 2000) and cinnamic glycosides such as rosin, rosavin, and rosarin (Zapesochnaya and Kurkin, 1982; Kurkin et al., 1985b, 1986; Satsyperova et al., 1993; Furmanowa et al., 1999a) have been identified. Other important constituents from rose root are flavonoids (Kurkin et al., 1982; Zapesochnaya and Kurkin, 1983; Zapesochnaya et al., 1985), tannins (Revina et al., 1976; Nekratova et al., 1992), gallic acid and its esters (Dubichev et al., 1991; Satsyperova et al., 1993) and essential oil (Kurkin et al., 1985a; Shirokov et al., 1980; Belov et al., 1994).

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The aim of the present study was to characterize the composition of terpenes and aroma volatiles from rhizomes of *Rhodiola rosea* as a potential source for large-scale production of essential oils and flavour essences. The investigation was partly perfomed within the framework of the project "Norwegian Herb Production (NUP) " in the period 1995–1998, which focused on the cultivation, improvement and marketing of medicinal plants and herbs in Norway. Samples from both steam distillation (SD) and headspace solid-phase microextraction (HS-SPME) have been analysed by gas chromatography/mass spectrometry (GC–MS). HS-SPME, introduced by Zhang and Pawliszyn (1993), has been widely applied in environmental research for the detection of air pollutants, water impurities and pesticides (Chai and Pawliszyn, 1995; Santos et al., 1996; Magdic et al., 1996). A considerable number of applications in the field of food chemistry and quality control was established which focussed on aroma volatiles (orange juice: Steffen and Pawliszyn, 1996; wine: Garcia et al., 1998; coffee: Roberts et al., 2000) and terpenes of essential oils from different sources (Schafer et al., 1995; Czerwinski et al., 1996; Fuchs et al., 1999; Paniandy et al., 2000). The HS-SPME technique has to a great extent been successfully introduced in the author's laboratory for biochemical and plant physiological-related applications (Rohloff, 1999; Rohloff et al., 2000a,b, 2002).

In the present study, HS-SPME has been applied to gain detailed information about the terpenic and aroma volatile composition of *Rhodiola rosea* rhizomes from Norway, and to identify and quantify the specific compounds responsible for the characteristic rose scent. To the best of the author's knowledge, a detailed investigation of the aroma volatiles from rose root has not yet been undertaken.

2. Results and discussion

The steam distillation of 100 g of raw plant material yielded a clear and colourless essential oil (0.05% of dry wt.) in contrast to results from Shirokov and co-workers (1980) who found an average content of 1%. With regard to other well-known terpene-containing root drugs from different plant families such as Compositae, Umbelliferae, Zingiberaceae and Araceae with oil contents from 0.5 up to 10% (Wichtl, 1997), the yield from rose root was rather low. However, the characteristic site of terpene synthesis and accumulation in higher plants is often restricted to aerial plant organs rather than to roots and rhizomes (Hay and Svoboda, 1993). An analysis task on cultivated rose root plants from Norway carried out at the Plant Biocentre in 2001 showed that the average oil content was below 0.05% (Dragland, 2001), and thus, underscores the presented data.

In total, 75 compounds were identified in the essential oil from the SD samples on the basis of a mass spectrum database search and retention indices (see Table 1), while 59 compounds were detected by peak area measurement (94.74% identified). In contrast, only 69 peaks were identified by the use of HS-SPME, and a total of 36 compounds was detected. The main chemical classes of volatile compounds were monoterpene hydrocarbons, monoterpene alcohols and straight chain aliphatic alcohols (see Table 2) which accounted for more than 86% of the essential oil. The most abundant compound was decanol (30.38%), which also was the main peak detected by HS-SPME. Other important constituents were geraniol (12.49%), 1,4-*p*-menthadien-7-ol (5.10%), limonene (4.90%), α-pinene (4.69%) and dodecanol (3.67%). In an investigation carried out at the author's laboratory on cultivated plant material from Norway (Dragland, 2001), monoterpenes (geraniol, myrtenol, pinocarveol) as well as alipathic (heptanol) and aromatic alcohols (cinnamyl alcohol and cuminyl alcohol) were found to be the main compounds in *Rhodiola* essential oil and showed similarities to the present study. In contrast to results reported by a Russian research group (Belov et al., 1994), the proposed main constituents such as octadecadienoic acid, heptanol derivates and hexadecanoic acid were only detected in insignificant amounts in the Norwegian samples.

Regarding the different analytical methods for the isolation of the volatile compounds, SD followed by GC–MS revealed a higher number of identified and detected peaks compared to HS-SPME coupled with GC–MS. With regard to the chosen extraction methods (conventional SD and HS-SPME), neither the monoterpene alcohol glycoside rosiridin nor its aglycon rosiridol found in rose root by Kurkin and co-workers (1985a), could be isolated. As already pointed out by Field et al. (1996), Coleman and Lawrence (1997), and Rohloff (1999), the applicability of SPME for terpene extraction is intimately linked to the parameters of sampling time and temperature conditions. In addition, it is influenced by the chosen fibre type and its diameter (Schafer et al., 1995). Depending on the applied method, the affinity of the non-polar and semi-polar terpenes and volatiles to the fibre type differs greatly, i.e. analysis of the headspace gas by SPME will under no circumstances render results from essential oil analysis of solvent-based samples (Rohloff, 1999). Although the number of detected compounds in distillates might be higher than those reported for sesqiterpenes for example, the soft and non-invasive SPME represents an analytical tool for the identification of additional volatiles not being isolated by SD (Rohloff, 1999; Paniandy et al., 2000; Beaulieu and Grimm, 2001). On the background of the number of identified compounds from essential oil and HS-SPME samples (75 versus 69, respectively), the presented results underscore the excel-

Table 1
Terpenes and headspace volatiles from rhizomes of *Rhodiola rosea* ordered by retention time from SD samples

No.	Compound	RA[a] (SD)	RT[b]	RA (HS-SPME)	RT
1	Santene	0.84	1.92	–	–
2	Tricyclene	0.36	2.07	–	–
3	α-Pinene	4.69	2.15	t	3.65
4	Camphene	0.91	2.43	t	4.85
5	Hexanal	0.05	2.57	1,54	5.53
6	β-Pinene	1.47	2.83	t	7.20
7	Sabinene	1.45	2.97	t	7.23
8	3-Carene	2.04	3.30	t	7.77
9	β-Myrcene	2.25	3.48	t	8.78
10	α-Terpinene	0.81	3.73	t	8.98
11	2-Heptanone	–	–	t	9.30
12	Heptanal	t[c]	3.82	t	9.47
13	Limonene	4.91	4.03	0,38	9.77
14	β-Phellandrene	2.31	4.18	t	9.95
15	2-Pentyl furan	0.08	4.65	0,54	11.65
16	*cis*-Ocimene	0.21	4.72	t	11.93
17	γ-Terpinene	1.83	4.92	–	–
18	*trans*-Ocimene	0.24	5.08	–	–
19	Styrene	0.06	5.18	1,73	12.43
20	*n*-Pentanol	t	5.35	t	12.70
21	*p*-Cymene	2.97	5.47	0,85	12.95
22	Terpinolene	0.42	5.77	t	13.53
23	*n*-Octanal	–	–	t	14.05
24	6-Methyl-5-hepten-2-one	0.03	7.30	0,54	16.27
25	*n*-Hexanol	0.03	7.90	t	17.33
26	*n*-Nonanal	t	9.17	0,77	18.70
27	Ocimenone	–	–	1,24	19.32
28	*trans*-2-Octenal	–	–	t	20.02
29	β-Thujone	t	10.62	–	–
30	β,*p*-Dimethyl styrene	t	10.72	0,52	20.40
31	*cis*-Linalool oxide	0.15	10.93	0,77	20.70
32	Menthone	0.44	11.45	1,28	21.03
33	*trans*-Linalool oxide	0.05	12.12	0,46	21.95
34	Isomenthone	0.12	12.57	t	22.18
35	*n*-Decanal	0.13	13.43	1,17	23.23
36	Benzaldehyde	0.12	14.18	2,07	23.82
37	Isopinocamphone	0.10	14.77	t	24.22
38	*trans*-2-Nonenal	0.03	14.90	t	24.43
39	Pinocarvone	0.05	15.87	t	25.08
40	Linalool	2.31	16.18	1,37	25.42
41	*n*-Octanol	2.77	16.60	3,66	25.67
42	Bornyl acetate	t	16.80	–	–
43	β-Caryophyllene	t	17.28	t	26.07
44	Neomenthol	–	–	0,29	26.58
45	Myrtenal	–	–	0,91	26.90
46	Menthol	0.08	20.28	t	27.68
47	*trans*-Pinocarveol	0.54	20.65	1,61	27.90
48	Neral	t	20.92	0,27	28.50
49	Estragol	0.10	21.43	–	–
50	*n*-Nonanol	0.72	22.52	2,09	28.68
51	α-terpineol	0.40	23.15	0,25	29.00
52	Geranyl formate	–	–	2,90	29.25
53	Carvone	0.15	24.03	t	29.50
54	Geranial	0.07	24.63	0,62	29.65
55	Geranyl acetate	0.11	26.38	t	30.25
56	Cuminyl aldehyde	0.35	26.50	–	–
57	Perilla aldehyde	0.09	26.70	t	30.62
58	*n*-Decanol	30.38	27.12	10,53	30.47
59	Myrtenol	1.73	27.88	1,45	30.92

(*continued on next page*)

Table 1 (*continued*)

No.	Compound	RA[a] (SD)	RT[b]	RA (HS-SPME)	RT
60	Nerol	0.07	28.77	–	–
61	*trans*-Carveol	t	29.85	–	–
62	*cis*-Carveol	0.10	30.32	–	–
63	Geraniol	12.49	31.37	t	31.87
64	Hexanoic acid	–	–	0,55	32.17
65	Benzyl alcohol	–	–	4,88	32.50
66	Octadecyl acetate	0.06	33.07	0,17	32.62
67	Phenylethyl alcohol	t	33.88	2,29	33.05
68	Cuminyl acetate	0.11	36.43	0,31	33.87
69	Dodecanol	3.67	37.42	0,66	33.98
70	Perilla alcohol	0.45	38.47	–	–
71	Cinnamaldehyde	0.15	39.33	3,67	34.98
72	Benzene propanol	0.09	40.47	0,47	35.17
73	1,4-*p*-Menthadien-7-ol	5.10	40.98	–	–
74	Octanoic acid	t	42.32	t	35.53
75	Cumin alcohol	2.66	43.17	1,44	35.98
76	Cinnamyl acetate	0.12	45.10	t	36.65
77	Thymol	t	47.90	–	–
78	Carvacrol	t	48.95	–	–
79	Cinnamyl alcohol	0.28	51.35	3,70	38.53
80	Decanoic acid	0.32	52.00	–	–
81	Dodecanoic acid	0.12	60.95	t	41.22
82	Acetovanillone	–	–	t	42.33
83	Benzyl benzoate	t	64.55	–	–
84	Tetradecanoic acid	t	69.25	t	44.85
85	Pentadecanoic acid	–	–	t	47.42
86	Hexadecanoic acid	t	77.13	t	50.80

[a] Relative area in % (peak area relative to total peak area).
[b] Retention time in minutes.
[c] Trace compound.

Table 2
Composition of the essential oil from SD samples of *Rhodiola rosea* rhizomes ordered by chemical groups (summarized peak area in %)

Compound group	
Monoterpene hydrocarbons	25.40
Oxygenated monoterpenes	
Alcohols	23.61
Aldehydes	0.29
Ketones	0.86
Straight chain alipathic compounds	
Alcohols	37.54
Aldehydes	0.08
Ketones	0.03
Acids + esters	0.50
Aromatic compounds	3.94
Phenols	0.10
O-Heterocyclic compounds	2.39
Total	94.74

lent suitability of solid-phase micro-extraction for qualitative analyses.

With regard to the characteristic rose fragrance of the rhizomes of *Rhodiola rosea*, several compounds with rose odour and other floral notes have been identified (see Table 1). Geraniol was identified as the main rose-like odour compound, which is one of the most abundant monoterpene alcohols in the essential oil from *Rosa* sp. (Lawless, 1996). Minor important constituents such as geranyl formate and geranyl acetate, benzyl alcohol and phenylethyl alcohol were also detected. Hegnauer (1964, 1989) relates also rose scent to the aglycon tyrosol, but from the presented data it can not be concluded in which way this phenolic compound contributes to the appearance of rose root fragrance in relation to geraniol.

Floral notes such as linalool and its oxides, nonanal, decanal, nerol and cinnamyl alcohol emphasize the flowery scent of rose root rhizomes, while alipathic alcohols such as decanol and dodecanol may be responsible for the fat- or wax-like background scent. Regarding the low oil yield and the diverging composition of terpenes and volatiles in rose root rhizomes, the applicability of essential oil or extracts from rose root in cosmetic and perfume industries will be rather restricted to the exclusive production for the national market. On the other hand, the present study reports only results from a local variety originating from Mid-Norway, and in fact, further investigation of this wide-spread mountain plant

might supplement and broaden the knowledge of the biochemistry of *Rhodiola rosea*.

3. Experimental

3.1. Plant material

R. rosea roots (5 kg fr. wt) were collected from wild growing plants at Alvdal, Norway (10°37/62°07) in September 1998. The roots were cut into small segments (1–2 cm), dried at 35 °C in a drying cabinet with fan (Termaks TS 5410) for 48 h and stored at room temperature prior to analysis in December 1998. Five samples each were used for extraction by steam distillation and SPME, respectively.

3.2. Steam distillation (SD)

The distillation apparatus consisted of a heating cap, a 3 l extraction flask, a 3 ml graduated receiver (Dean and Stark) and a condenser (jacketed coil). One-hundred grams of dried plant material and 1.5 l H_2O were used and the distillation was carried out for 3 h after reaching the boiling point. The collected oil was directly used for sample preparation by diluting 10 μl oil in 1 ml ethanol in brown autosampler flasks.

3.3. Headspace solid-phase micro-extraction (HS-SPME)

A PDMS coated fiber (100 μm) and a manual SPME holder (SUPELCO Inc.) were used for sample extraction. In a blank run, the fibre was exposed to the GC inlet for 3 min for thermal desorption at 250 °C before headspace sampling. One gram of each sample was sealed in a 10 ml screw top vial with phenolic cap and PTFE/ silicone septa (SUPELCO Inc.) and stored in a drying cabinet at 50 °C for 15 min. The SPME fibre was exposed to each sample for 1 min by manually penetrating the septum (0.25 cm depth).

3.4. Gas chromatography–mass spectrometry (GC–MS)

Analyses were carried out by using a Varian Star 3400CX gas chromatograph coupled with a Saturn 3 mass spectrometer. The capillary column was a Chrompack CP-Wax 52CB (30 m × 0.32 mm ID, 0.25 μm film thickness), and the carrier gas was He (5 psi). *Oil samples*—the initial oven temperature was 60 °C, rising at 2 °C min^{-1} to 210 °C and then held isothermal for 5 min. The injector, transfer line and detector temperatures were 220 °C, 210 and 175 °C, respectively. Samples were injected by splitless injection. *SPME samples*—the SPME fibre was inserted into the injection port of the GC for 2 min for sample desorption. The oven temperature was held isothermal at 35 °C for 5 min, programmed from 35 to 90 °C at 3 °C min^{-1}, subsequently at 7 °C min^{-1} up to 210 °C and then held isothermal for 15 min. The injector, transfer line and detector temperatures were 240, 210 and 175 °C, respectively. Samples were injected by using the split-sampling technique with a ratio of 1:27.5. All mass spectra were acquired in EI mode (scan range $m/z = 40$–300, 5 μscans s^{-1}, multiplier voltage 1700 V, ionization energy 70 eV). Chromatogram peaks were identified by mass spectra database search (VARIAN NIST MS Database 1992 and IMS Terpene Library 1992) and on the basis of relative retention indices (ESO 00, Database of Essential Oils, BACIS 1999). Quantitative analysis (in%) was performed by peak area measurement (TIC).

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