AROMA OF SOME PLANTS CULTIVATED IN LITHUANIA:

COMPOSITION, PROCESSING AND RELEASE

Eglė Bylaitė

Promotors: Dr. Ir. A.G.J. Voragen
                 Hoogleraar in de levensmiddelenchemie

Co-promotoren: Dr. Ir. R.P. Venskutonis
                 Universitair hoofddocent
                 departement Levensmiddelentechnologie, Technische Universiteit Kaunas,
                 Litouwen

                 Dr. Ir. J.P. Roozen
                 Universitair hoofddocent
                 departement Agrotechnologie and Voedingswetenschappen

AROMA OF SOME PLANTS CULTIVATED IN LITHUANIA:

COMPOSITION, PROCESSING AND RELEASE

Eglė Bylaitė

Proefschrift
ter verkrijging van de graad van doctor
op gezag van de rector magnificus
van Wageningen Universiteit
Dr C.M. Karssen,
in het openbaar te verdedigen
op vrijdag 9 Juni 2000
des namiddags te half twee in de Aula.

Bylaitė, Eglė

Aroma of some plants cultivated in Lithuania: composition, processing and release

Thesis Wageningen University- With Summary in Dutch

Abstract

Bylaitė, E. (2000). Aroma of some plants cultivated in Lithuania: composition, processing and release. Ph.D. thesis, Wageningen University and Research Centre.

Keywords: aroma plants, essential oils, Umbelliferae, Asteraceae, harvesting time, dynamic headspace, olfactometry, essential oil emulsification, microencapsulation, milk proteins, flavour release, microstructure.

In this study, some factors affecting the aroma of some plants of the families *Umbelliferae* and *Asteraceae* were evaluated. The composition of the aromas is influenced by several factors: plant family, harvesting time, anatomical part of plant, method used to isolate volatiles, cultivar, fertilisers used for the growing of plant, cultivation site. The yield of caraway fruits varies over a wide range depending on fertiliser content, cultivation area and the cultivar itself. The trends for the accumulation of essential oils differ between the families in yield and composition. Seeds and flowers of lovage (*Umbelliferae*) possess the highest yields of oil. In the leaves of lovage seasonal changes are less significant than in the stems. In leaves and flowers of costmary (*Asteraceae*) the highest oil content is obtained before full flowering, while their stems possess only negligible amount of volatiles. Various anatomical parts of lovage showed differences in flavour release measured by the dynamic headspace method. The effluents from a gas chromatography column were characterised by a sniffing panel, which attributed descriptors to the recognised constituents.

Liquid essential oils can be processed by emulsification and encapsulation with milk proteins. Stability of essential oil-in-water emulsions can be improved either by adding soybean phosphatidylcholine and/or by increasing its protein concentration. Adsorption of the protein from the aqueous phase at the oil/water interface was studied by applying ellipsometry. Essential oil of caraway was encapsulated by milk derivatives either alone or combined with carbohydrates. Partial replacement of whey protein concentrate increases the retention of volatiles during spray drying and enhances the protective properties of solidified capsules against oxidation and the release of volatiles during the timed period. The structural features of spray- dried capsules indicated that good physical protection is provided to the caraway essential oil.

CONTENTS

Chapter   **Page**

Propositions / Stellingen

1. The accumulation of essential oil in the plant depend on growing conditions and anatomical part the of plant.
   (Perry, N.B. et al.(1999). Essential oils from Dalmatian sage (*Salvia officinalis* L.): variations among individuals, plant parts, seasons and sites. *J. Agric. Food Chem.* 47, 2048–2054.This thesis.)

2. Optimal yield of essential oil relates to the harvesting time for various species of plants.
   (Mallavarapu, G.R. et al. (1999). Influence of plant growth stage on the e ssential oil content and composition in davana (*Artemisia pallens* Wall.). *J. Agric. Food Chem.* 47, 254–258. Senatore, F (1996). Influence of harvesting time on yield and composition of the essential oil of a thyme (*Thymus pulegioides* L) growing wild in Campania (southern Italy). *J. Agric. Food Chem.* 44, 1327–1332. This thesis.

3. Ellipsometry is the best way to measure an adsorption of β-lactoglobulin at the caraway essential oil/water interface.
   (This thesis).

4. A smaller droplet size of emulsions correlates with a higher retention of essential oil and with a smaller amount of surface oil after spray drying.
   (Rish, S.J.; Reineccius, G.A (1988). Spray-dried orange oil: effect of emulsion size on flavour retention and shelf stability. In *Flavour encapsulation* , eds S.J. Rish, G.A. Reineccius. ACS symposium series 370; American Chemical Society: Washington, D.C.; pp 67–77).

5. Phthalides play a major role in the aroma of celery and lovage.
   (Uhlig, J.W. et al. (1987) Effect of phthalides on Celery flavour. *J. Food Sci* . 52 (3), 658–660. This thesis).

6. Aromas can be stabilised by encapsulating them into skim milk powder.
   (This thesis)

7. Shelf-life of essential oils is more related to the exposure of light than to temperature change.
   (This thesis)

8. Use of food supplements may lead to health problems.

9. The development of electronic communication technologies leads to a decline of communication between people.

10. It's better to do nothing than to be busy doing nothing.
    (L.N.Tolstoj)

11. Integration of Lithuania into EC for its economic stability can give a risk to loose its identity.

12. A conclusion is the place where you got tired of thinking.
   (S. Wright)

Propositions belonging to the thesis by Eglė Bylaitė entitled
Aroma of some plants cultivated in Lithuania: composition, processing and release
Wageningen, 9th of June 2000

CHAPTER 1

GENERAL INTRODUCTION

FLAVOUR

The flavour of food can be defined as a complex sensation primarily composed of aroma and taste but also complemented by tactile and temperature stimuli (Heath and Reineccius, 1986). Taste is concerned with sensations of the tongue in response to salty, sweet, sour and bitter. The tongue surface also reacts to tactile and temperature stimuli, which include the cooling of menthol and the heat of red pepper. There is a tactile response to texture, astringency, etc. – all of which contribute to the overall perception of food flavour. Aroma is a much broader sensation, coming from a small area in the nasal passage and encompassing an estimated 10 000 or more different odours (Reineccius, 1994).
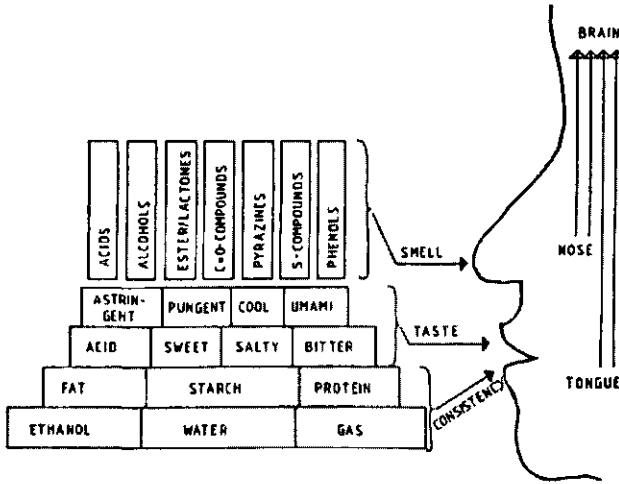
Fig 1.1. A schematic diagram categorising the key components of flavour in what is described as an aromagram. Each food consists of various combinations of the boxes from the three levels of smell, taste, and consistency. In addition, there will be a variety of personal, social, and cultural factors (from Ney, 1990).

A novel visual conceptualisation of flavour was presented by Ney (1990) using a schematic diagram that consists of a series of boxes on three main levels (Fig. 1.1). The top level consists of boxes related to smell, including monocarboxyl acids, alcohols, esters, lactones, carbonyl compounds, pyrazines, sulphur and phenol chemicals. The middle (double) layer of boxes consists of taste components, incorporating the trigeminal aspects of astringency,

pungency, coolness and umami in the uppermost row and acid, sweet, salty, bitter in the lower row. Along the bottom (also double) layers Ney has a series of boxes with the overall label "consistency". The upper boxes of the bottom layer are fat, starches and proteins. The lower layer consists of ethanol, water and gases (mainly CO_2) which are the media in which the above components will be found.

NATURAL FLAVOURS: PLANTS AS A SOURCE OF NATURAL FLAVOURINGS

Flavours are considered to be natural if they are obtained exclusively by physical or fermentative methods from natural original materials (Jones, 1982). Examples of physical methods are pressing, distillation, extraction, concentration and chromatography. Fermentative methods are understood to cover the formation of flavouring agents by enzyme catalysis, microorganisms and plant cell cultures. The fermentative methods are also known as biotechnological methods.

Flavouring materials of natural origin can be defined as follows (Heath and Reineccius, 1986):

- *Natural aromatic raw materials.* Plants, vegetable and /or animal products used for their flavouring properties, either as such or as processed for human consumption
- *Natural flavours.* Concentrated preparations obtained exclusively by physical means from natural aromatic raw materials
- *Natural flavouring substances.* Substances isolated from natural aromatic raw materials exclusively by physical means.

Even now, plants still remain the main universal source for making natural flavourings of food grade. Besides their flavourful properties, they also contain nutrients. With careful choice, plants can provide a quite adequate balance of the protein, carbohydrates, fats, vitamins and mineral acids required for a healthy diet (Heath, 1981). However, many plants are more valuable for their aromatic properties, the spectrum of which is immense. Some must be regarded as fragrances, whereas others are primarily of value as flavourants, but in the creation of imitation flavourings and fragrance compounds the demarcation is very imprecise. Hodge and Bailey (1975) reviewed the history and botanical classification of flavour-producing plants, the broad

extent of which emphasises the problems of classification of sensory attributes and the difficulty of selecting those of major interest to the flavourist.

Plant material contains both volatile and non-volatile constituents, which affect their odour and/or flavour profile as well as their sensory impact. The volatile constituents give the plant its distinctive odour whereas the non-volatile constituents are either inert (e.g. cellulose) or influence some gustatory reaction (e.g. bitterness, pungency, astringency, etc.) sometimes coupled with a physiological effect, e.g. coffee, cocoa leaves (Fisher and Scott, 1997). The intensity and quality of these effects show very wide variations between plant families and species. Although, the specific aromatic profiles remain recognisable and within acceptable limits, a quantitative variation is often observed between different crops and batches of the same plant material and also between the several parts of the plant that may be used (Huopalahti and Linko, 1983; Bylaite et al.; 1996; Fiorini et al.; 1997; Arganosa et al., 1998; Perry et al., 1999; Munne-Bosch et al., 2000).

PROCESSING OF AROMA PLANTS

The excessively wetness of freshly harvested herbs makes their shelf life and application as flavouring material rather limited. To extend their seasonal availability and to have them in the most convenient form for handling, most of this plant material requires further processing. The degree of finesse of the processed aroma of herbs and spices is determined by its ultimate use (e.g. distillation, extraction, blended seasonings) (Heath and Reineccius, 1986).

The most common ways to process plant material are the following:

Drying

Domestically, fresh herbs are widely used in the form of a *bouquet garni, fines herbes*, etc., as these have a better aroma and flavour than the dried forms. However, on a commercial scale, the dried form is much easier to handle.

To achieve a stable product, the water content must be reduced from about 60–80% in the fresh herb to 5–10% in the dried. If this is carried out under optimum conditions the colour of the herb is retained and flavour losses are reduced to a minimum (Jaganmohan-Rao et al., 1998; Bartley and Jacobs, 2000). Excessive heat results in an unacceptable degree of browning, the loss of freshness and the imposition of off-odour which detracts from the overall profile (Masanetz and Grosch, 1998; Masanetz et al., 1998). Most herbs are dried naturally in the shade but artificial drying at a maximum temperature of 40°C and also freeze-drying are increasingly being used.

Comminution

To make herbs and spices easier to incorporate directly into food products, most of them require processing such as commination, which is also an essential first stage in the extraction or distillation process. The reduction in particle size enables solvent or steam to penetrate and come into close contact with the cellular tissues containing the active constituents (Murthy et al., 1999). Comminution can directly affect the quality and keeping properties of the resulting material in the following ways:

- by exposing the material to high temperature during the grinding process
- by exposing the volatile oil in ruptured cellular tissues to losses by evaporation and/or oxidative changes
- by altering the physical character of the product, thereby affecting its subsequent shelf life and value as a flavouring material.

Elimination of microbiological spoilage

Herbs and spices are particularly susceptible to bacteria, spores, moulds and yeasts, some of which may be pathogenic or toxigenic (Krischnaswamy et al., 1973). For food processing, microbiological spoilage can be significantly alleviated or eliminated by the following processes:

- exposure of herbs to sterilant gases or gamma-irradiation
- sterilisation by heat processing
- distillation and/or extraction techniques to isolate the flavouring components of the spice: obtaining essential oils and extracts.

ESSENTIAL OILS

Essential oils, also known as essences or volatile oils, are complex mixtures of volatile substances located in the plant within distinctive oil cells (Lawrence, 1995). Resulting from the distillation essential oils are basically free of bacterial contamination and form the basis of a whole range of products for direct incorporation into food mixes. Moreover, some of them possess antimicrobiological activity (Rafiq-Siddiqui et al., 1996; Sivropoulu et al., 1997; Lis-Balchin et al., 1998; Mazzanti et al., 1998; Delaquis et al., 1999).

Constituents of essential oils include hydrocarbons and their oxygenated derivatives which comprise alcohols, acids, esters, aldehydes, ketones, amines, sulphur compounds, etc. Mono-, sesqui- and even diterpenes constitute the composition of a majority of essential oils. In addition, the phenyl propanoids, fatty acids and their esters are also encountered in a number of essential oils. These products give to the plant its distinctive and often diagnostic odour (Fisher and Scott, 1997). Their nature and relative proportions are determined by the plant species and agricultural factors such as environment, climate, soil conditions, time of harvesting and post-harvesting handling prior to distillation (Heath, 1981; Hussien, 1995; Chalcat et al., 1997; Chang-Hwan-Cho et al., 1997; Mallavarapu et al., 1999; Miraldi, 1999; Sefidkon et al., 1999; Schaller and Schnitzler, 2000).

Essential oils used in flavourings can be categorised as follows (Lawrence and Shu, 1993):
- *Common herbs* (sage, rosemary, marjoram, basil, oregano, thyme, parsley, spearmint, peppermint)
- *Spices* (black pepper, nutmeg, clove bud, ginger, cardamom)
- *Aromatic seeds/fruit* (anise, caraway, cumin, coriander, carrot, celery, dill, parsley)
- *Aromatic roots* (angelica, lovage, valerian)
- *Citrus* (orange, lemon, lime, grapefruit, bergamot, mandarin)
- *Conifers* (balsam fir, pine needle, cedar leaf)
- *Fragrant herbs* (lavender, melissa, hyssop, geranium, tagetes, Roman camomile)
- *Tree leaves* (clove, cinnamon, laurel, eucalyptus, lemon, mandarin)
- *Bark* (massoia, cascarilla)
- *Wood* (rosewood, sandalwood, cedar)

- *Florals*   (rose, jasmine, mimosa, cassie)
- *Grass*   (lemongrass, palmarosa, citronella)
- *Buds/seeds/fruits* and other organs star anise, clove stem, juniper berry, blackcurrant bud, hop)

Another way of looking at these oils is to categorise them according to whether they are rich or poor in monoterpene hydrocarbons, sesquiterpene hydrocarbons, oxygenated constituents, phenols or phenol esters. Finally they can be grouped together according to their major component (Lawrence and Shu, 1993):

- 1,8- cineole rich oils
- Eugenol rich
- Anethole rich
- Linalool/linalyl acetate rich
- Carvone rich
- Methyl chavicol rich
- Carvacrol/thymol

They define four classification systems depending on the application:

- *Botanical*   (depending either upon plant form or on botanical family associations)
- *Agronomic*   (based on the nature and extent of cultivation)
- *Physiological*   (based on the tradition and widespread use of natural plant materials as remedies)
- *Organoleptic*   (based on sensory attributes which are related to the prime aromatic constituents or in some cases non-volatile components)

Table 1.1 shows a selection of raw materials used for natural flavours. They are obtained by physical or fermentative methods.

Among the traditional raw materials, the isolates are growing increasingly more important. Table 1.2 shows some examples of isolates from essential oils. These are single chemical substances.

Table 1.1. A selection of raw materials for natural flavours (Arctander, 1960; Pollock, 1984).

Raw materials	Remarks
Essential oils	Pressing or distillation (steam distillation) of plant/ vegetable matter
Extracts	
Oleoresins	Extracts from plant / vegetable matter containing essential oil and resin as well as exudates from plants
Tinctures and percolates	Ethanolic extracts
Concretes	Extracts with organic solvents
Absolutes	Ethanolic extracts of concretes and other extracts
Concentrates	Concentrated extracts and juices
Distillates	Distilled extracts and plant/vegetable matter (e.g. ethanolic distillation)
Isolates	
Terpene-free or terpene- and sesquiterpene-free oil	Essential oils from the majority of terpenes and sesquiterpenes obtained by distillation, extraction or chromatography
Terpenes	By- product of obtaining terpene-free oils
Recombined essential oils	Recombination of various fractions of essential oils obtained by physical methods
Biotechnologically produced substances	
Complex mixtures	Produced by micro-organisms, enzymes, plant cell cultures or by storing foodstuffs in atmospheres containing flavour precursors
Single chemical substances	Produced by enzymes or physically isolated from the above mixtures
CO_2 extracts	Extraction from vegetable matter with supercritical carbon dioxide

Among the novel raw materials that can be produced biotechnologically, currently only those obtained by using enzymes are of any practical importance. However, the essential oils remain the main material for the preparation of natural flavourings.

Table 1.2. Examples of isolates from essential oils (single chemical substances).

Isolate	Original substance
Eugenol	Oil of cloves
Linalool	Rosewood oil
Geraniol	Palmarosa oil
Methyl-methyl anthranilate	Petitgrain mandarin oil

Apart from the advantages of being hygienic, free from enzymes, and possessing good flavour quality consistent with the source raw material; the essential oils also exhibit some disadvantages such as difficult handling because of their concentrated, liquid and often viscous state, their tendency to oxidate too readily, and not being easily dispersible, particularly in dry products. To avoid these problems and facilitate better handling, the essential oils can be further processed in order to obtain dry, free-flowing solid state products. This requires that the liquid flavour chemicals are either adsorbed on a dry carrier or encapsulated in inert edible polymers. The most common way of obtaining solid flavourings is by encapsulation.

ENCAPSULATION

Many food products for which development was thought to be technically unfeasible are made possible today because of the wide availability of encapsulated ingredients. Such ingredients are products of a process that totally envelops the ingredient in a coating or "capsule", thereby conferring many useful and otherwise unusual properties to the original ingredient. In a broad sense, encapsulation technology includes the coating of minute particles of ingredients (e.g. acidulants, fats, and flavours) as well as whole ingredients (e.g. raisins, nuts and confectionery products), which may be accomplished by microencapsulation and macro-coating techniques, respectively. It can also be defined as a physical process where thin films or polymer coats are applied to small solid particles, droplets of liquids or gases (Bakan, 1973). The material coated or entrapped is referred to by various names, such as core material, payload, actives or internal phase. The material forming the coating is referred to as the wall material, carrier, membrane, shell or coating. Excellent reviews of microencapsulation technology have been written, as applied to foods (Balassa and Fanger, 1971; Dziezak, 1988; Jackson and Lee, 1991; Gibbs et al., 1999) and to food flavours (Reineccius, 1989, 1991; Benczedi and Blake, 1999).

Aromas, as a rule, are complex mixtures of more or less volatile substances and labile components which can change as a result of oxidation, chemical interactions or vaporisation. In order to minimise the danger of this happening, microencapsulation processes are widely used in the flavour industry to entrap liquid flavouring substances in a carrier matrix and convert them into dry, free-flowing materials. In addition, protecting against external influences (oxidation, water, light and so on), reducing the volatility of the flavouring substances (better storability),

microencapsulated dry products facilitate easier handling in the application and, by selection of the correct carrier matrix, they play a crucial role in making some applications possible at all. With some encapsulation techniques, the product can be designed to either release slowly over time or to release at a certain point (Reineccius, 1995; Tuley, 1998; Benczedi and Blake, 1999). Encapsulation can be also used to separate components of a flavouring that react with each other such as acetaldehyde and methyl anthranilate.

Overall flavouring costs can be reduced in comparison to "liquid applications" as a result of clearly improved storability and a certain amount of control over the release of flavour in the application.

To select the "right" microencapsulation process, the following points should be examined and taken into account from the outset (Eckert, 1995):

- type of raw materials employed (natural, nature-identical, artificial)
- what auxiliary substances are incorporated (solvents, carrier substances)
- solubility of the liquid flavour (water soluble, oil soluble)
- world-wide legislation, food regulations, requirements and legality; application-specific processing parameters which the microencapsulated flavour must withstand
- when and how aromatisation takes place
- flavour-release mechanism
- requirements regarding particle size, bulk density and storability
- dosage guidelines for the selection of the optimal flavour loading
- price guidelines/aromatising costs.

Principles and techniques

There are many techniques that can be used to microencapsulate food ingredients. The selection of the method depends on economics, sensitivity of core, size of microcapsules desired, physical/chemical properties of both core and coating, applications for the food ingredient and the release mechanism.

While the encapsulation techniques used in the industry are numerous, spray drying and extrusion are the two major commercial processes in terms of product volume. Minor techniques would include freeze drying, coacervation, fat or wax encapsulation and inclusion in cyclodextrins.

Spray drying

Spray drying is the oldest commercial technique for producing encapsulated flavourings (Reineccius, 1988). Food ingredients entrapped by this method also include fats, oils and flavour compounds (Re-Mi, 1998). In addition to being an encapsulation process, spray drying is also a dehydration process and is used in the preparation of dried materials such as powdered milk. The spray-drying process involves three basic steps:

1. Preparation of dispersion or emulsion to be processed
2. Homogenisation of the dispersion
3. Atomisation of the mass into a drying chamber.

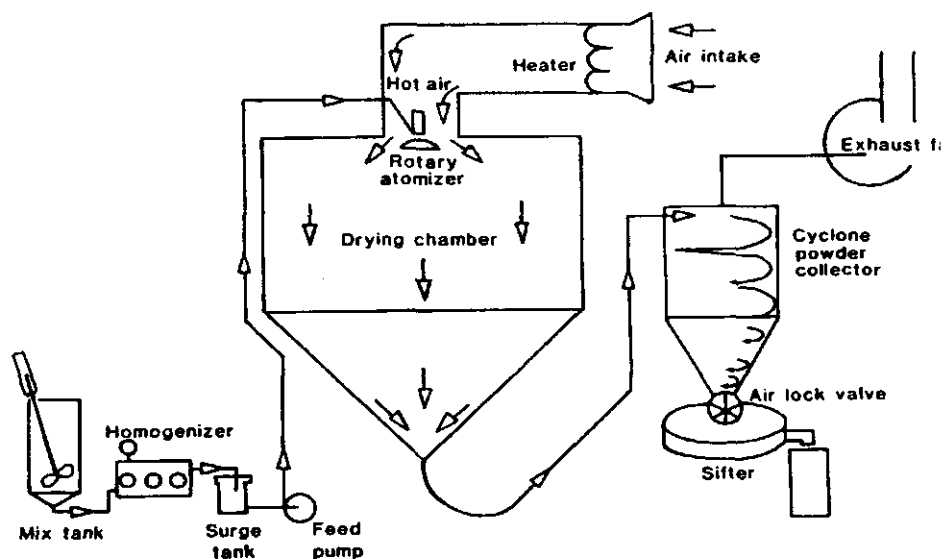A typical system used in spray drying is displayed in Fig. 1.2.

Fig. 1.2: Typical system used in spray -drying.

The material to be atomised is prepared by dispersing an active (core) material, usually a flavour or oil, into a solution of the coating (wall) with which it is immiscible. The most generally used wall materials for the encapsulation of food flavours by spray drying are vegetable gums (gum acacia, gum traganth), starches, modified starches, dextrins, proteins and sugars (Balassa and Fanger, 1971). A typical ratio of carrier to core material is 4:1; however, in some applications higher flavour loads can be used.

Following the addition of an emulsifier, the mixture is homogenised to give an oil-in-water type of emulsion and to create small droplets of flavour or ingredient within the carrier solution. Risch and Reineccius (1988) have shown that there is a direct relationship between degree of homogenisation and the retention of orange peel oil during spray drying and shelf-stability of spray-dried products (Table 1.3).

Table 1.3. Influence of emulsion size on the retention of orange oil during spray drying (from Risch and Reineccius, 1988)

Sample	Total oil content (g oil/ 100 g powder)
Coarse	13.8
Medium coarse	17.3
Medium fine	18.6
Homogenised	19.1
Microfluidised	20.0

The results of this study indicated that there are advantages to creating smaller emulsion droplets when preparing solutions for spray drying even though larger or coarser emulsions are much easier to prepare and require less sophisticated equipment. The main advantage of smaller emulsions is a better retention of citrus oil in the spray-dried powder. A second advantage is that smaller emulsions also yield dried powders which have less extractable surface oil. A third advantage of producing a finer emulsion is that the emulsion is more stable (Risch and Reineccius, 1988). This is particularly important for beverage applications where viscosity cannot be increased to help in stabilising the flavour emulsion.

The core/wall material is fed into a spray dryer in which it is atomised through a nozzle or spinning wheel into a heated airstream supplied to the drying chamber. As the atomised particles fall through the gaseous medium, they assume a spherical shape with the oil encased in the aqueous phase. This explains why most spray-dried particles are water-soluble. The rapid evaporation of water from coating during its solidification keeps the core temperature below 100°C. The particles are exposed to heat for a few seconds at most. Thus, the main advantage of this method is its ability to handle many heat-labile materials. However, because any material such as a flavour may contain as many as 20–30 different components (alcohols, aldehydes, esters and ketones) with boiling points ranging from 38°C to 180°C, it is possible to lose those aromatics that have a low boiling point during the drying process (Taylor, 1983). The choice of drying inlet and exit air temperatures is determined primarily by the carrier matrix and flavour being dried. Some natural flavouring materials (e.g. cheese, tomato purée) may suffer from heat damage when dried using high exit air temperatures. In terms of flavour retention, high exit air temperatures have been found to be beneficial to the retention of water-soluble flavours (Reineccius and Coulter, 1969).

The advantages of spray drying include low processing costs and readily available equipment. It generally provides good protection to the core material and there is a wide variety of wall material available. One main disadvantage is that it produces a very fine powder which needs further processing, such as agglomeration, to instantise the dried material or to make it more readily soluble if it is for a liquid application (Risch, 1995). Due to the heat required for evaporation of water from the system, spray drying is not good for heat-sensitive materials (King 1990; Jackson and Lee, 1991).

A modification of the spray drying process that is suitable for encapsulating highly volatile or thermolabile substances is called the "cold dehydration process", which was proposed by Zilberboim et al. (1986a). Here, an emulsion of core material in coating material solution is sprayed into a dehydrating liquid such as ethanol or polyglycols at room temperature or below. The microcapsules are recovered by filtration and vacuum dried at a low temperature.

Additional work by Zilberboim et al. (1986b) studied the microcapsules produced by this technique in an attempt to determine the effects of different process parameters on retention and

shelf life. This method does provide an alternative to the high temperatures encountered in spray drying; however, the lack of easily available equipment to accomplish it in a continuous flow instead of as a batch operation makes it much more costly than spray drying. There are no significant commercial applications of this procedure; however, it does provide an alternative for expensive, heat-labile materials where the additional cost might be justified.

A modification of spray drying – the Leaflash spray dryer – has been proposed by Bhandari et al (1992). In this drying technique, the hot air flowing at a very high velocity in the converging section of the dryer head atomises and simultaneously dries the resulting atomised droplets. The vibration and turbulent rotational motion of the droplets caused by impact of whirling hot air during atomisation enhances the transfer rate. Thus, drying time is reduced and it is possible to use a much higher inlet air temperature (i.e. 450°C). The enhanced drying rate also permits the use of a dryer chamber of reduced volume. Moreover, this atomising system is capable of atomising the liquid at relatively high viscosities, which permits an increase in the total solids content in the feed (Bhandari et al., 1992).

Extrusion

Extrusion is the second most frequently used process, after spray drying, for encapsulating flavours (Reineccius, 1989; Gunning et al., 1999). Encapsulation by extrusion involves dispersion of the core material in a molten carbohydrate mass (Risch and Reineccius, 1988). This mixture is forced through a die into a dehydrating liquid which hardens the coating to trap the core material. The strands or filaments of hardened material are broken into small pieces, separated and dried. A typical process involves mixing flavour materials with hot corn syrup or modified starch (120°C) and extruding the mixture as pellets into a cold solvent bath such as isopropanol. The cold solvent solidifies the syrup into an amorphous solid and washes residual flavour oil from the surface. Capsules obtained by this technique give a good protection of the flavour, which usually represents 10–25% of the product mass

Coacervation

Microencapsulation by coacervation is not extensively used by the food industry since it is complicated and expensive. However, most scientists consider it the true microencapsulation

process in that the wall material completely surrounds the core with a continuous coating of wall material (Soper, 1995). Very few food grade polymers are available (gum arabic, gelatin) for use as coatings. Microencapsulation by coacervation involves three steps. First, three immiscible phases: the continuous phase (water), the material to be encapsulated and the coating material are mixed (Blenford, 1986). In the second step, the coating is deposited on the core material. This is accomplished by changing pH, temperature or ionic strength which results in a phase separation (coacervation) of the coating and its entrapment of dispersed core (Bakan, 1973). Finally, the coating is solidified by thermal, crosslinking or desolvant techniques.

Inclusion complexation

Inclusion complexation is the only method of encapsulation that takes place on a molecular level. It uses β-cyclodextrin to complex and entrap molecules (Hedges et al., 1995; Bhandari et al., 1998, 1999; Yoshii et al., 1998). Cyclodextrins were used to stabilise emulsions and foams and to protect sensitive food ingredients from light, heat and oxygen. Cyclodextrins consist of 7 glucose units linked 1-4 and are formed by enzymatic degradation of corn starch with alpha amylase, followed by treatment with the enzyme cyclodextrin transglycosylase. The functional properties of cyclodextrins are conferred by the difference in the hydrophobicities between the centre and outside of the molecule. The outer surface of cylodextrin is hydrophilic in nature due to hydroxyl groups. The centre of cyclodextrin is hydrophobic as it is lined with glycosidic oxygen bridges. Compounds able to form inclusion complexes with cyclodextrins are hydrophobic or have hydrophobic side groups. The hydrophobic portion of the guest molecule forms stable non-covalent interactions with the centre of the cyclodextrins. This complex becomes less soluble and will precipitate out of solution. (Saenger, 1980).

Summarising the description of microencapsulation processes for flavours and their applications in the food industry, it becomes clear, that there are still many starting points for further intensive research and development in this field. These will be aimed at improving existing processes and making new ones available, thus being able better to adapt and optimise the additive "flavour" for both existing and new uses.

Table 1.4: Examples of applications of microencapsulated flavours

Products	Applications
Lemon flavour emulsified	Expanded sugar goods, gums
Lemon compound	Drinks
Punch-cloudicol flavouring	
Strawberry flavour spray-dried	
Condensed milk flavour spray-dried	Expanded sugar goods
Rum flavour spray-dried	
Red wine flavour natural spray-dried	Desserts
White wine flavour natural spray-dried	Instant sauces
Nutmeg extract natural spray-dried	Instant sauces
Black pepper extract natural spray-dried	Instant soups
Thyme extract natural spray-dried	
Banana-Neorome fruit powder spray-dried	Instant baby foods
Strawberry-Neorome fruit powder spray-dried	Diet products
Orange-Neorome fruit powder spray-dried	Waffle fillings
Amaretto flavour vacuum dried	Instant coffees
Green soup vegetable extract mixture, vacuum dried	Instant soups
Cyclodextrin products (e.g. garlic oil in cyclodextrin)	At present pharmaceutical products
Colouring foodstuffs	Pasta
Spray-dried spinach	
Spray-dried turbidifying solids (food colour)	Drinks powders
+Tang type orange-flavour, spray-dried	

FLAVOUR ISOLATION AND ANALYSIS

Normally the concentration of aromatic compounds in food is very low. Only in such aroma-rich products as spices, herbs, distilled alcoholic beverages and coffee does the sum of volatiles exceed 0.1% (Rothe, 1988). The techniques applied for the recovery of volatiles have to meet the following important criteria: (1) all compounds making an important contribution to a certain flavour should be extracted in the correct proportions, (2) the techniques applied should not alter

the structure of key aromatic compounds and (3) non-volatile compounds which could interfere with the gas chromatographic separation should be removed completely (Engel et al., 1999).

The method most often applied to recover volatiles from plant material is distillation, which uses the high volatility of aromatic compounds and dates back to the ninth century. Although the actual process has not changed much over the centuries, the method used has been refined and modified to give optimum yields under conditions which can be controlled. Actual yields and quality depend on the nature of the feed materials, the methods used in their handling, pre-treatment and speed of processing after comminution. Modern distillation techniques comprise water, steam, water and steam categories. Generally water distillation gives the finest quality oils as with this technique there is less chance of damage to sensitive components of the essential oil and giving a burnt character which can result from internal condensation and flowback of an aqueous extract onto the exposed steam injection coils (Heath and Reineccius, 1986). The advantage of distillation is complete separation of all non-volatile components. They are normally present in higher amounts than the volatiles, which would be expected to interfere with component isolation and subsequent identification (Lawrence and Shu, 1993).

To ensure that all volatiles are recovered, the combined simultaneous steam and organic solvent distillation using Likens-Nickerson apparatus or similar can be used. However each distillation technique has its disadvantage. Steam distilled samples are boiling, so destruction of components as well as formation of artefacts is possible (Weurman, 1969; Fisher et al., 1988; Schieberle, 1995). Recently, a compact and versatile distillation unit has been developed for the fast and safe isolation of volatiles from complex food matrices (Engel et al., 1999). In connection with a high vacuum pump, the new technique, designated solvent assisted flavour evaporation (SAFE), allows the isolation of volatiles from either solvent extracts, aqueous foods or even matrices with a high oil content free from non-volatile matrix compounds. Thus, the method of isolating volatiles has to be chosen very carefully.

Steam or water distilled essential oils do not necessarily reflect the composition of the actual odour of an aromatic plant. Essential oils are mostly artefacts formed during the distillation (Baser, 1995). The headspace technique aims at capturing the real odoriferous compounds of a plant material. It can be applied to live or cut flowers and to anything that smells. Static

headspace provides an equilibrium of compounds which are perceived by odour and contributes to the identification of volatile constituents which are detected by a human nose above the food (Guth and Grosch, 1993). However, the method is limited to the level of detection and identification of organic volatiles and especially semi-volatiles (Arino et al., 1999). Relatively low sensitivity is achieved with this method due to the limited volume of headspace gas to be injected into the gas chromatograph. The detection threshold capabilities of GC detectors and mass spectrometers prevent the detection of trace levels of odours often present in headspace despite the fact that these compounds are often odour-significant even at low concentrations. Attempts to increase sensitivity by injecting larger amounts of headspace gas result in loss of chromatographic resolution due to the inability to focus the large injection volume in a relatively narrow chomatographic zone. This invariably results in severe peak broadening and loss of sensitivity (Hartman et al., 1993). To concentrate volatiles prior to the injection into the gas chromatograph, the dynamic headspace technique DHS is currently being used. This technique is more sensitive and permits a wider range of analysis of volatiles. It involves sweeping of the volatiles released from the food with a stream of purified carrier gas such as helium or nitrogen and trapping them onto adsorbents such as Tenax, charcoal or by cryogenic means (Morton and MacLeod, 1982). The volatiles trapped onto adsorbents can be injected to GC by thermal desorption or by eluting them with proper solvent from the adsorbent. In this way, the sensitivity of recovering volatiles can be increased, permitting the analysis of volatiles present at the parts per billion (ppb) level routinely.

The identities of many of the individual volatile components present in food aroma isolates can be established rapidly by GC–MS analysis. However, the complexity of the volatiles from many foodstuffs is such that important components, especially those present in trace amounts, are often either poorly separated from or totally masked by other compounds. Consequently, the interpretation of their mass spectra may be very difficult. It is possible to apply a variety of procedures, of which some serve to reduce the complexity of the sample and facilitate final identification by GC–MS while others provide a great deal of independent information not readily obtained by other means.

The GC technique is very important for aroma analysis and the power of both spectroscopic and chemical methods of identification is most effectively exploited when allied to its high separating power and sensitivity.

Progress in instrumental analysis has led to a long list of volatiles. Unfortunately, the sensory relevance of these volatile compounds has not been extensively evaluated by identifying impact compounds. Therefore, one of the major problems in aroma research is to select those compounds, which significantly contribute to the aroma of a food. In general, the aroma of a food consists of many volatile compounds, and only a few them are sensorially relevant (Blank, 1997). GC in combination with olfactometric techniques (GC-O) is a valuable method for the selection of aroma-active components from a complex mixture (Grosch, 1993). Experiments based on characterising the odours of single compounds emerging from the GC sniffing ports by volunteers, are described as GC-O or GC sniffing. This technique helps to detect potent odorants without knowing their chemical structures. Experience shows that many key aroma compounds occur at very low concentrations; their sensory relevance is due to low odour thresholds. Thus, the peak profile obtained by GC-FID does not necessarily reflect the aroma profile of a food (Blank, 1997). In GC-O, the human nose is the detector used for evaluating the effluent of the GC.

FLAVOUR RELEASE

The driving force for the flavour release of volatile compounds is the deflection from the thermodynamic equilibrium between the product phase and the gas phase. Equilibrium exists if the concentration in the respective phases obey the relation:

$$K_{gp} = C_g/C_p$$

where K_{gp} is the equilibrium partition coefficient between gas and product phase, which is determined by the volatility and solubility of the flavour compound. C_g and C_p represent the concentrations in the gas and product phase, respectively. As flavour compounds are usually presented in highly diluted solutions, C_g and C_p are related by Henry's law when equilibrium

exists. If the actual partition coefficient is smaller than K_{gp}, flavour will be released (Overbosch et al., 1991).

Besides the partition coefficient, the resistance to mass transfer is a major factor determining the rate and extent of flavour release. Partitioning of flavour compounds is affected by the composition of the food (Druaux and Voilley, 1997). Non-equilibrium is the driving force for mass transport and the rate at which equilibrium is achieved, is determined by the resistance to mass transfer (De Roos and Wolswinkel, 1994). Most studies in this area dealt with partition phenomena; in particular with the effect of medium composition on the equilibrium headspace concentrations, but they were limited to simplified food models. The first studies on the partitioning of volatile compounds between air and water were conducted by Buttery et al. (1969, 1971). They reported that, of all food components, lipids affect equilibrium headspace concentrations most. Consequently, the investigations on the threshold concentrations of aromatic compounds have showed that aroma efficacy is lower in a fat medium than in aqueous systems (Guadagni et al., 1972). A mathematical model developed by Harrison and Hills (1997) to describe flavour release from aqueous solutions containing flavour-binding polymers suggested that in most experimental situations, the rate-limiting step for flavour release is not the chemical binding step but the transport of aroma across the liquid/gas interface. Bakker et al. (1998) confirmed that transfer of diacetyl flavour molecules across the liquid–gas interface is rate limiting and is adequately described by the penetration theory of mass transfer.

Tyszkiewicz and Jackowska (1982) have demonstrated a relationship between concentration and odour intensity of caraway extract in different media. They found large differences in the threshold values. In comparison with lipids, hydrophilic food components revealed frequently a limited effect on the concentrations of flavour compounds in the headspace over aqueous solutions. The presence of proteins (Overbosch, 1991) resulted in weak unspecific hydrophobic interactions only. Studies on the interactions occurring between volatile compounds and hydrocolloids showed that the addition of xanthan and guar gum lowered flavour release compared to that from water (Yven et al., 1998).

It is clear that flavour release from certain products depends not only on the interactions between different aroma components but also on the medium of the product. So, one should expect that

products with a different state (dried herbs, essential oils, oils encapsulated to different media), even though they possess the same flavour constituents, will exhibit different dynamics and rates of flavour release. This aspect should be taken into account for prediction of odour perception and control of aroma in the final products.

SCOPE OF THE THESIS

The objective of the study described in this thesis was to evaluate the flavour of aromatic plants grown and cultivated in Lithuania and to examine some aspects of flavour release and processing, i.e. emulsification and encapsulation.

For this purpose, a number of plants with different chemical composition were selected for the isolation and investigation of their volatile constituents. Furthermore, some factors such as harvesting time, fertilisation, variety and anatomical part of the plant were also investigated because of their influence on yield, and on the qualitative and quantitative composition of essential oils in plants.

Investigations into the chemical composition of volatiles of different plants and the factors that have an influence on volatile composition are described in **Chapters 2, 3** and **4**. Two plants (lovage and caraway) were chosen for further examination: lovage because of conflicting data on its odorant characteristics; and caraway because of the suitability of its chemical composition for modelling studies. **Chapter 5** deals with comparisons of flavour composition and release in different isolation conditions: the isolation of volatiles was achieved by DHS. In addition to gas chromatography and mass spectrometry, olfactometric analysis (gas chromatography–sniffing port) has also been used to determine the aroma of lovage. **Chapters 6** and **7** present studies on the application of caraway essential oil for preparing and obtaining flavourings. The formation of oil-in-water emulsions with flavour substances and the properties of the caraway oil-in-water interfaces thus formed are described in **Chapter 6**. Finally, in **Chapter 7**, the complex behaviour and properties of encapsulated caraway oil products are described, together with the microstructural features of capsules.

REFERENCES

Acree, T. E.; Barnard, J. Gas chromatography-olfactometry and Charm Analysis. In *Trends in Flavour Research*; Maarse, H., Van der Heij, D. G., Eds.; Elsevier Science Publishers B. V.: Amsterdam, 1994; pp 211-220.

Arctander, S. *Perfume and flavour materials of natural origin*. Arctander Publication, Elizabeth, NJ, USA, 1960.

Arganosa, G.C.; Sosulski, F.W.; Slikard, A.E. Seed yields and essential oil of northern-grown coriander (*Coriandrum sativum* L.). *J. Herbs Spices Med. Plants* **1998**, 6, 23–32.

Arino, A.; Arberas, I.; Renobales, G.; Dominguez, J.B. Influence of extraction method and storage conditions on the volatile oil of wormwood (*Artemisia absinthium* L.). *Eur. Food Res. Technol.* **1999**, 209, 126–129.

Bakan, J. A. Microencapsulation of foods and related products. *Food Technol.* **1973**, 27, 11–44.

Bakker, J.; Boudaud, N.; Harrison, M. Dynamic release of diacetyl from liquid gelatin in the headspace. *J. Agric. Food Chem.* **1998**, 46, 2714–2720.

Balassa, L.L.; Fanger, G.O. Microencapsulation in the food industry. *CRC Critical Reviews in Food Technology* **1971**, July, 245–263.

Bartley, J.P.; Jacobs, A. Effects of drying on flavour compounds in Australian-grown ginger (*Zingeber officinale*). *J. Sci. Food Agric.* **2000**, 80, 209–215.

Baser, H.C.K. Analysis and quality assessment of essential oils. In *A manual on the essential oil industry*, Tuley de Silva (ed.); United Nations industrial development organization, Vienna, 1995; pp 155–178.

Benczedi, D.; Blake, A. Encapsulation and the controlled release of flavours. *Leatherhead Food RA Food Ind. J.* **1999**, 2, 36–47.

Bhandari, B.R.; Dumoilin, E.D.; Richard, H.M.J.; Noleau, I.; Lebert, A.M. Flavour encapsulation by spray drying: application to citral and linalyl acetate. *J. Food Sci.* **1992**, 57, 217–221.

Bhandari, B.R.; D'Arcy, B.R.; Bich, L.L.T. Lemon oil to β-cyclodextrin ratio effect on the inclusion efficiency of β-cyclodextrin and the retention of oil volatiles in the complex. *J. Agric. Food Chem.* **1998**, 46, 1494–1499.

Bhandari, B.R.; D'Arcy B.R.; Padukka, I. Encapsulation of lemon oil by paste method using β-cyclodextrin: encapsulation efficiency and profile of oil volatiles. *J. Agric. Food Chem.* **1999**, 47, 5194–5197.

Blank, I. Gas chromatography – olfactometry in food aroma analysis. In *Techniques for analyzing food aroma;* Marsili, R. (ed.), Marcel Dekker, Inc. New York, 1997; pp 293–330.

Blenford, D. Fully protected. *Food Flavor Ingred. Proc. Pckg.* **1986**, July, 43.

Buttery, R.G.; Ling, L.C.; Guadagni, D.G. Food volatiles: volatilities of aldehydes, ketones and esters in dilute water solution. *J. Agric. Food Chem.* **1969**, 17, 385–389.

Buttery, R.G.; Bomben, J.L.; Guadagni, D.G; Ling, L.C. Some considerations of the volatilities of organic flavour compounds in foods. *J. Agric. Food Chem.* **1971**, 19, 1045–1048.

Bylaitė, E.; Legger, A.; Roozen, J. P.; Venskutonis, P. R. Dynamic headspace gas chromatography of different botanical parts of lovage (*Levisticum officinale* Koch.). In *Developments in flavour science;* Taylor, A. J., Mottram, D. S. (eds.); The Royal Society of Chemistry, UK, 1996; pp 66–69.

Chalcat, J.C.; Michet, A.; Pasquier, B. Influence of harvesting time on chemical composition of *Mentha piperita* L. essential oil. *Perfum. Flavor.* **1997**, 22, 17–21.

Chang-Hwan-Cho; Jong-Chul-Lee; Young-Hyun-Choi; Ouk-Kyu-Han. Effects of temperature, light intensity and soil moisture on growth, yield and essential oil content in valerian (*Valeriana fauriei* var. *dasycarpa* Hara). *Korean J. Crop Sci.* **1997**, 42, 22–32.

Delaquis, P.J.; Ward, S.M.; Holley, R.A.; Cliff, M.C.; Mazza, G. Microbiological. chemical and sensory properties of pre-cooked roast beef preserved with horseradish essential oil. *J. Food Sci.* **1999**, 64, 519–524.

De Roos K.B.; Wolswinkel K. Non-equilibrium partition model for predicting flavour release in the mouth. In *Trends in flavour research*, eds. H. Maarse, D.G. van der Heij. Elsevier Science, Amsterdam, The Netherlands, 1994; pp 15–32.

Druaux, C.; Voilley, A. Effect of food composition and microstructure on volatile flavour release. *Trends Food Sci. Technol.* **1997**, 8, 364–368.

Dziezak, J.D. Microencapsulation and encapsulated ingredients. *Food Technol.* **1988**, 42, 136–151.

Eckert, M. Microencapsulated flavours: manufacture and possible applications. *Dragoco Report. Flavouring Information Service* **1995**, 1, 5–19.

Engel, W.; Bahr, W.; Schieberle, P. Solvent assisted flavour evaporation – a new and versatile technique for the careful and direct isolation of aroma compounds from complex food matrices. *Eur. Food Res. Technol.* **1999**, 209, 237–241.

Fiorini, C.; Fouraste, I.; David, B.; Bessiere, J.M. Composition of the flower, leaf and stem essential oils from *Laurus nobilis* L. *Flavour Fragrance J.* **1997**, 12, 91–93.

Fisher, C.; Scott, T.R. *Food flavours: biology and chemistry*. The Royal Society of Chemistry, 1997.

Fisher, N.; Nitz, S.; Drawert, F. Original flavour compounds of marjoram flavour and its changes during processing. *J. Agric. Food Chem.* **1988**, 36, 996–1003.

Gibbs, B.F.; Kermasha, S.; Alli, I.; Mulligan, C.N. Encapsulation in the food industry: a review. *Int. J. Food Sci. Nutr.* **1999**, 50, 213–224.

Grosch, W. Detection of potent odorants in foods by aroma extract dilution analysis. *Trends Food Sci. Technol.* **1993**, 4–68.

Guadagni, D.G; Buttery, R.G.; Turnbaugh, J.G. Odour thresholds and similarity ratings of some potato chip components. *J. Sci. Food Agric.* **1972**, 23, 1435–1444.

Gunning, Y.M.; Gunning, P.; Kemsley, E.K.; Parker, R.; Ring, S.G.; Wilson, R.H.; Blake, A. Factors affecting the release of flavor encapsulated in carbohydrate matrixes. *J. Agric. Food Chem.* **1999**, 47, 5198–5205.

Guth, H.; Grosch, W. Identification of potent odourants in static headspace samples of green and black tea powders on the basis of aroma extract dilution analysis (AEDA). *Flavour Fragrance J.* **1993**, 8, 173–178.

Harrison, M.; Hills, B.P. Mathematical model of flavor release from liquids containing aroma binding macromolecules. *J. Agric. Food Chem.* **1997**, 45, 1883–1890.

Hartman, T.G.; Lech, J.; Karmas, K.; Salinas, J.; Rosen, R.T.; Ho, C.T. Flavour characterization using adsorbent trapping–thermal desorption or direct thermal desorption–gas chromatography and gas chromatography–mass spectrometry. In *Flavour measurement*; Ho, C.; Manley, C.M. (eds.); Marcel Dekker Inc., New York, 1993; pp 267–328.

Heath, H.B.; Reineccius, G.A. *Flavour chemistry and technology*. Macmillan Publishers USA 1986.

Heath, H.B. *Source book of flavours*. AVI Publishing Company Inc., USA, 1981.

Hedges, A.R.; Shieh, W.J.; Sikorski, C.T. Use of cyclodextrins for encapsulation in the use and treatment of food products. In *Encapsulation and controlled release of food ingredients*, eds S.J. Risch, G.A. Reineccius. ACS symposium series 590; American Chemical Society: Washington, D.C., 1995; pp 60–71.

Hodge, W.H; Bailey, L.H. Survey of flavour producing plants. *Flavour Ind.* **1975**, 6, 244-245.

Huopalahti, R.; Linko, R.R. Composition and content of aroma compound in dill, *Anethum graveolens* L., at three different growth stages. *J. Agric. Food Chem.* **1983**, 31, 331–333.

Hussien, M.S. Response of growth, yield and essential oil of coriander and dill to different nitrogen sources. *Egyptian J. Horticulture* **1995**, 22, 1–10.

Jackson, L.S.; Lee, K. Microencapsulation in food industry. *Lebensm.-Wiss. u.-Technol.* **1991**, 24, 289–297.

Jaganmohan-Rao, L.; Meenakshi, S.; Raghavan, B.; Abraham, K.O. Rosemary (*Rosmarinus officinalis* L.): impact of drying on its flavor quality. *J. Food Quality* **1998**, 21, 107–115.

Jones, K. Verordnung zur Neuordnung lebensmittelrechtlicher Kennzeichnungsvorschriften. *Dragoco Bericht* **1982**, 27, 31–36.

King, C.J. Spray drying food liquids and the retention of volatiles. *Chem. Eng. Prog.* **1990**, 86, 33–39.

Krischnaswamy, M.A.; Patel, J.D.; Parthasarathy, N.J.; Nair, K.K. Microbiological quality of certain spices. *J. Plantation Crops* **1973**, 1 (Suppl.), 200.

Lawrence, B.M. The isolation of aromatic materials from natural plant products. In *A manual on the essential oil industry;* Tuley de Silva (ed.); United Nations Industrial Development Organization, Vienna, 1995; pp 57–154.

Lawrence, B.M.; Shu, Ch. Essential oils as components of mixtures: analysis and differentiation. In *Flavour measurement*; Ho, C.; Manley, C.M. (eds.); Marcel Dekker Inc., New York, 1993; pp 267–328.

Lis-Balchin, M.; Buchbauer, G.; Hirtenlehner, T.; Resch, M. Antimicrobial activity of Pelargonium essential oils added to a quiche filling as a model food system. *Letters in Applied Microbiology* **1998**, 27, 207–210.

Mallavarapu, G.R.; Kulkarni, R.N.; Baskaran, K.; Rao, L.; Ramesh, S. Influence of plant growth stage on the essential oil content and composition in davana (*Artemisia pallens* Wall.). *J. Agric. Food Chem.* **1999**, 47, 254–258.

Masanetz, C.; Grosch, W. Hay-like off flavour of dry parsley. *Z. Lebensm.-Unters. Forsch.* **1998**, 206, 114–120.

Masanetz, C.; Guth, H.; Grosch, W. Fishy and hay-like off flavours of dry spinach. *Z. Lebensm.-Unters. Forsch.* **1998**, 206, 108–113.

Mazzanti, G.; Battinelli, L.; Salvatore, G. Antimicrobial properties of the linalol-rich essential oil of *Hyssopus officinalis* L. var *decumbens* (Lamiaceae). *Flavour Fragrance J.* **1998**, 13, 289–294.

Miraldi, E. Comparison of the essential oils from ten *Foeniculum vulgare* Miller samples of fruits of different origin. *Flavour Fragrance J.* **1999**, 14, 379–382.

Morton, I.D.; MacLeod, A.J. *Food flavours*. Part A. Introduction. Elsevier Scientific Publishing Company, 1982.

Munne-Bosch, S.; Alegre, L.; Schwarz, K. The formation of phenolic diterpenes in *Rosmarinus officinalis* L. under Mediterranean climate. *Eur. Food Res. Technol.* **2000**, 210, 263–267.

Murthy, C.T.; Meenakshi, R.; Srinivasa, R.P.N. Optimal grinding characteristics of black pepper for essential oil yield. *J. Food Process Eng.* **1999**, 22, 161–173.

Ney, K.H. Aromagrams: a new approach to flavour classification and description. In *Flavours and Off-Flavours*, Charalambours, G. (Ed.), Elsevier Science, Amsterdam, 1992.

Overbosch, P.; Achterof, W.G.M.; Haring, P.G.M. Flavour release in the mouth. *Food Rev. Int.* **1991**, 7, 137–187.

Perry, N.B.; Anderson, R.E.; Brennan, N.J.; Douglas, M.H.; Heaney, A.J.; McGimpsey, J.A.; Smallfield, B.M. Essential oils from Dalmatian sage (*Salvia officinalis* L.): variations among individuals, plant parts, seasons and sites. *J. Agric. Food Chem.* **1999**, 47, 2048–2054.

Pollock, C.L. Natural raw materials. What they are; how they are used in flavour creation. In *The development and application of natural and artificial flavour systems*. Allured Publishing, Wheaton IL, USA, 1984; pp 47–63.

Rafiq-Siddiqui, R.; Hamid-Ahmad; Sultan-Shakoor, C.; Ehteshamuddin, A.F.M.; Safina-Shireen. Antimicrobial activity of essential oils. II. *Pakistan J. Scient. Ind. Res.* **1996**, 39, 43–47.

Reineccius, G.A. Spray drying of food flavours. In *Flavour encapsulation*, eds S.J. Risch, G.A. Reineccius. ACS symposium series 370; American Chemical Society: Washington, D.C., 1988; pp 57–66.

Reineccius, G. A. Flavour encapsulation. *Food Rev. Int.* **1989**, 5, 147–176.

Reineccius, G.A. Carbohydrates for flavor encapsulation. *Food Technol.* **1991**, March, 144-149.

Reineccius, G. A. *Source book of flavors.* Chapman and Hall, New York, 1994.

Reineccius, G.A. Controlled release techniques in the food industry. In *Encapsulation and controlled release of food ingredients*, eds S.J. Risch, G.A. Reineccius. ACS symposium series 590; American Chemical Society: Washington, D.C., 1995; pp 9–25.

Reineccius, G. A.; Coulter, S.T. Flavour retention during drying. *J. Dairy Sci.* **1969**, 52, 12–19.

Re - Mi. Microencapsulation by spray drying. *Drying Technol.* **1998**, 1195–1236.

Risch, S.J.; Reineccius, G.A. Spray-dried orange oil: effect of emulsion size on flavour retention and shelf stability. In *Flavour encapsulation*, eds S.J. Risch, G.A. Reineccius. ACS symposium series 370; American Chemical Society: Washington, D.C., 1988; pp 67–77.

Risch, S.J. Encapsulation: overview of uses and techniques. In *Encapsulation and controlled release of food ingredients*, eds S.J. Risch, G.A. Reineccius. ACS symposium series 590; American Chemical Society: Washington, D.C., 1995; pp 2–7.

Rothe, M. In *Introduction to aroma research.* Rothe, M. (ed.) Kluwer Academic Publishers, Dordrecht, The Netherlands, 1988; pp 30–35.

Saenger, W. Cyclodextrin inclusion compounds in research and industry. *Angew Chem. Int. Ed. Engl.* **1980**, 19, 344–362.

Schaller, R.G.; Schnitzler, W.H. Nitrogen nutrition and flavour compounds of carrots *(Daucus carota* L) cultivated in Mitscherlich pots. *J. Sci. Food Agric.* **2000**, 80, 49–56.

Schieberle, P. Quantitation of important roast-smelling odorants in popcorn by stable isotope dilution assays and model studies on flavor formation during popping. *J. Agric. Food Chem.* **1995**, 43, 2442–2448.

Sefidkon, F.; Dabiri, M.; Rahimi-Bidgoly, A. The effect of distillation methods and stage of plant growth on the essential oil content and composition of *Thymus kotschyanus* Boiss. & Hohen. *Flavour Fragrance J.* **1999**, 14, 405–408.

Sivropoulu, A.; Nikolaou, C.; Papanikolaou, E.; Kokkini, S.; Lanaras, T.; Arsenakis, M. Antimicrobial, cytotoxic and antiviral activities of Salvia fructicosa essential oil. *J. Agric. Food Chem.* **1997**, 45, 3197–3201.

Soper, J.C. Utilization of coacervated flavors. In *Encapsulation and controlled release of food ingredients*, eds S.J. Risch, G.A. Reineccius. ACS symposium series 590; American Chemical Society: Washington, D.C., 1995; pp 104–112.

Tyszkiewicz, I.; Jackowska, A. Technological applications of the results of odour stimulus threshold determination in the example of caraway. *Nahrung* **1982**, 26, 649-652.

Taylor, A.H. Encapsulation systems and their applications in the flavour industry. *Food Flavours, Ingredients, Packaging and Processing* **1983**, 5, 48–49, 51–52.

Tuley, L. Flavour in a bubble. *Food Manufacture* **1998**, 73, 26–28.

Weurman, C. Isolation and concentration of volatiles in food odor research. *J. Agric. Food Chem.* **1969**, 17, 370–384.

Yoshii, H.; Furuta, T.; Okita, E.; Toyomi, A.; Linko, Y.Y.; Linko, P. The increased effect of kneading on the formation of inclusion complexes between d-limonene and β-cyclodextrin at low water content. *Biosci. Biotech. Biochem.* **1998**, 62, 464–468.

Yven, C.; Guichard, E.; Giboreau, A.; Roberts, D. Assessment of interactions between hydrocolloids and flavor compounds by sensory, headspace, and binding methodologies. *J. Agric. Food Chem.* **1998**, 46, 1510–1514.

Zilberboim, R.; Kopelman, I.J.; Talmon, Y.J. Microencapsulation by a dehydrating liquid: retention of paprika oleoresin and aromatic esters. *J. Food Sci.* **1986**a, 51, 1301–1306.

Zilberboim, R.; Kopelman, I.J.; Talmon, Y.J. Microencapsulation by a dehydrating liquid: a microstructural study by scanning electron microscopy. *J. Food Sci.* **1986**b, 51, 1307–1310.

CHARACTERISATION OF THE ESSENTIAL OILS OF SOME

UMBELLIFEROUS PLANTS GROWN IN LITHUANIA

This chapter is based on the following articles:

1. Bylaitė, E.; Venskutonis, R. Essential oils of parsley and celery from Lithuania. Scientific works *"Food Chemistry and Technology"*, Vilnius "Academia", **1994**, 69–74.

2. Bylaitė, E.; Venskutonis, R. Volatile constituents of dill. Proceedings of the conference *"Maisto chemija ir technologija"*, Kaunas , "Technologija", **1994**, 15–18.

3. Venskutonis, R.; Kvietkauskaitė, D.; Bylaitė, E.; Šuliauskas, A. Characterization of caraway (*Carum carvi* L.) cultivated in Lithuania. *Horticulture and Vegetable Growing. Scientific Works of the Lithuanian Institute of Horticulture and Lithuanian University of Agriculture*, **1999**, 18 (3), 85–92.

ABSTRACT

The essential oils of parsley, celery, dill and caraway (family *Umbelliferae*) grown in Lithuania were characterised using GC and GC/MS analyses. The chemical composition of the aroma of locally grown parsley, celery and dill plants shows that the amounts of the major constituents are in agreement with the published data (Kasting et al., 1972; MacLeod et al., 1985; Halva et al., 1988; Cu et al., 1989; Badoc and Lamarti, 1991; Bouwmeester et al., 1995a,b,c). The most abundant components in locally grown parsley were found to be β-phellandrene (21%), myristicin (20%), myrcene (11%) and α, *p*-dimethylstyrene (11%). In terms of quantity, the main constituent of celery essential oil was limonene (63%) and 3-*n*-butylphthalide (6%) was the major phthalide. The dill herb could be ascribed to chemotype II (dillapiole), which is distinguishable by its high concentrations of limonene (27%), dill ether (21%) and D-carvone (14%), and the absence of myristicin.

The yield from seeds of different caraway cultivars, their essential oil content and chemical composition, were examined as well as the influence of fertilisers on those characteristics. Twelve batches of caraway fruits commercially produced in 1994, 1995 and 1996 in different regions of Lithuania were also characterised. Total concentration of essential oil in the fruits varied from 2 to 4 ml 100 g^{-1}. The yield of caraway fruits grown in the experimental fields varied over a wide range (from 984 to 2673 kg ha^{-1}) depending on fertiliser content and cultivation area and on the cultivar itself. Percentage concentrations of the main caraway compounds, limonene and carvone, varied in the ranges of 38–52% and 45–59%, respectively. These two compounds constituted more than 96% of the total essential oils in all samples. Some minor compounds were also identified and assessed quantitatively.

INTRODUCTION

Parsley, celery, dill and caraway (family *Umbelliferae*) are most popular aromatic plants in Lithuania and are widely used for flavouring and seasoning various foods. They can be used as fresh or frozen herbs, dried (herbs and seeds) or processed into more concentrated products

like essential oils. The essential oils of these plants are used in baked goods, pickled products, confectionery, chewing gums, condiments and beverages.

The chemical composition of the essential oil from parsley (*Petroselinum sativum* Hoffm.) leaves has previously been reported (Kasting et al., 1972; MacLeod et al., 1985). Fifty volatile compounds were identified in the headspace vapours of concentrates obtained by steam distillation and isopentane extraction from parsley leaves (Kasting et al., 1972). The main constituents were found to be myristicin, 1,3,8-*p*-menthatriene, β-phellandrene and myrcene. MacLeod et al. (1985) identified 45 compounds, of which 14 were present in amounts exceeding 1% of the total peak area: α-pinene, β-pinene, myrcene, limonene, β-phellandrene, *p*-cymene, α-terpinolene, 1,3,8-*p*-menthatriene, 4-isopropenyl-1-menthylbenzene, α-terpineol, *p*-methylacetophenone, α-elemene, apiol and myristicin. The presence of the unusual sesquiterpenes, crispanone and crispane, in parsley has been described by Appendino et al. (1998).

The volatiles of celery (*Apium graveolens* L.) leaves and seeds were first studied by Wilson (1969). Using GC/MS, Cu et al. (1989) detected 50 compounds, 27 of which were identified. Phthalides have been considered as substantial celery flavour constituents, but there is some confusion about their identification and quantification, especially of sedanolide and sedanenolide. Bjeldanes and Kim (1977) isolated two major phthalides from celery essential oil: 3-*n*-butylphthalide and 3-*n*-butyl-4,5-dihydrophthalide (sedanenolide). Later butylphthalide, sedanolide and sedanenolide were separated by HPLC (Uhlig et al., 1987) and by two-dimensional capillary GC (Van Wassenhove et al., 1988). Nine phthalides were found and *trans*-neocnidilide, sedanolide and butylphthalide were the main representatives. In 1997, Bartschat et al., using enantioselective multidimensional GC, achieved the simultaneous analysis of the three butylphthalide enantiomers, and their distributions in celery and celery seed extracts were elucidated.

Aroma composition of dill (*Anethum graveolens* L.) has been the subject of numerous investigations which, however, have resulted in rather controversial conclusions (Baslas and Baslas, 1972; Göckeritz et al., 1979; Lawrence, 1980b; Schreier et al., 1981). The amounts of the three main constituents (D-carvone, α-phellandrene, limonene) were reported to vary widely. Dill ether (3,6-dimethyl-2,3,3a,4,5,7a-hexahydrobenzofuran) was found to be a key

compound with respect to the aroma of dill (Brunke et al., 1991). The amounts of the main aroma compounds in dill at three different vegetative periods were found to vary within the following ranges: α-phellandrene 26–34%; limonene 4–10%; dill ether 11–32%; and D-carvone 0–3% (Huopalahti and Linko, 1983). The influence of the area of cultivation was studied by Halva et al. (1988) and it was determined that the contents of α-phellandrene, dill ether and myristicin can vary in ranges of 37–59%, 22–38% and 0.3–2% respectively. Badoc and Lamarti (1991) investigated the composition and the content of volatile compounds in essential oil depending on the chemotype of the herb. They reported that three broad chemotypes could be distinguished by the amount of D-carvone and the presence of myristicin and dill ether.

Caraway (*Carum carvi* L.) is the most important commercially cultivated spice in Lithuania. However, the effect of different factors on the yield of locally cultivated caraway seeds, the influence of different factors on the accumulation of essential oil and its chemical composition in this country have not yet been comprehensively examined. It is well established that the composition of caraway seed essential oil is very simple. Carvone (approx. 55% in the total oil) and limonene (approx. 45% in the total oil) are the major constituents, while the other components of the oil do not exceed 1% (Lawrence, 1980a; Formacek and Kubeczka, 1982; Hälvä et al., 1986; Analytical Methods Committee, 1988; Chalcat et al., 1991; Puschmann et al., 1992; Bouwmeester and Smid, 1995; Bouwmeester et al., 1995a,b, 1999). Puschmann et al. (1992) analysed caraway seeds from 16 different regions of origin and found that the content of essential oil was about 5%, of which carvone about 50% and limonene content about 45%. However, some exceptions have been observed, for example, El-Wakeil et al. (1986) determined 80% of carvone and only 9.8% of limonene in the essential oil of *Carum carvi* fruits from Egypt.

Carvone content in caraway essential oil is regulated by the standards: e.g., according to Hungarian regulations (Hungarian Standard, 1981) it has to be more than 50%; ISO standard (ISO, 1987) requires caraway oil to have not less than 48% of carbonyl compounds expressed as carvone; and in the British Pharmacopoeia (British Pharmacopoeia, 1988) the content of ketones is said to constitute 53–63% (w/w) calculated as carvone. The influence of different factors on caraway seed yield, the content of essential oil and its main constituents has been

studied thoroughly by Bouwmeester and Kuijpers (1993) and Bouwmeester et al. (1995a,b,c, 1998). For gas chromatographic (GC) analysis, caraway essential oil is usually isolated by hydrodistillation; however, Kallio et al. (1994) and Baysal and Starmans (1999) used the supercritical carbon dioxide extraction-GC method. In the study of Kallio et al. (1994) some differences in the concentrations of carvone and limonene as compared with those obtained with hydrodistillation were found. The ratio carvone:limonene in the CO_2 extracts obtained from three cultivars from different provenances used in the above mentioned study was greater than that obtained with hydrodistilled essential oils, while this ratio in the fourth cultivar was higher in the hydrodistilled oil than in the CO_2 extract. The composition of the hexane extracts was also different than that of hydrodistilled oils: the content of limonene was approximately 10–20% higher in the extracts than in the oils, whereas the content of carvone was higher in the oils (Bouwmeester et al., 1995a).

The chemical composition of plant aromas depends on various factors, such as harvest date (i.e. the state of maturity), geographical origin, chemotype, and isolation procedure of the aroma compounds (Heath, 1981). In general, the data published on the volatile composition of aromatic plants, particularly parsley, celery, dill and caraway, show that flavour profiles can differ significantly depending on the country of origin and conditions of cultivation of the plant (Haelvae et al., 1992a,b, 1993; Jirovetz et al., 1994; Hussien, 1995; Pino et al., 1995; Evers et al., 1997; Arganosa et al. 1998; Zawirska-Wojtasiak et al., 1998). Therefore, studies on the aromatic herbs and spices grown in different countries and/or climatic zones are always of interest. This research was undertaken for the aroma characterisation of most popular herbs grown in Lithuania: parsley, celery, dill and caraway.

MATERIALS AND METHODS

Steam distilled commercial essential oils of parsley, celery and dill leaves were obtained from a local company "Mėta". The essential oils were stored in a refrigerator before analysis. For direct GC/MS analysis the essential oils were dissolved in distilled ether: parsley and dill 0.2% (v/v), celery 0.1% (v/v). Commercially grown samples of caraway seeds (Skemiai-1'1994, Skemiai-2'1994, Skemiai' 1995, Meskuičiai' 1994, Panevėžys' 1995, Agrolitpa-

1'1995, Agrolitpa-2'1995, Agrolitpa-1'1996, Agrolitpa-2'1996, Vilkija' 1995, Kupiskis' 1996, Alytus' 1996) were obtained from different suppliers in different areas of Lithuania.

Experimental samples of four different cultivars (Gintaras, Rekord, Chmelnickij and Prochana) were grown in experimental fields at the Lithuanian Agricultural University in Kaunas region (fertilisation = 30 kg ha^{-1} NaNO$_3$). Fertilisation experiments were carried out for the cultivar Rekord in experimental fields in Klausučiai, Jurbarkas region. Nitrogen fertilisers were applied ranging in amount from 0 to 120 kg ha^{-1}. The fruits were harvested by hand in the first half of July and dried at 40°C to about 10% moisture and stored in paper bags at ambient temperature protected from light. Essential oils were isolated from 20 g of ground seeds by hydrodistillation (3–4 times) in a modified European Pharmacopoeia apparatus for 2h (the essential oil collecting tube was surrounded by a water cooling jacket) (Richard et al., 1992).

GC analyses

Essential oils from caraway samples were diluted in ether 0.2% (v/v) and analysed on two instruments:

1. For identification purposes on a dual column HP 5890A chromatograph with FID: split inlet 1:100; helium as carrier gas at inlet pressure 138 kPa and linear velocity 35 cm s^{-1}; fused silica dimethylpolysiloxane DB-1 and PEG DB-WAX columns, both 60 m length, 0.25 mm i.d. and 0.25 μm film thickness; temperature programming from 50 to 238°C (8 min hold) increasing at 4°C min^{-1}; injector temperature 220°C, detector temperature 260°C.

2. For quantification on a Fisons chromatograph with FID: split inlet 1:20; helium as carrier gas at inlet pressure 138 kPa and linear velocity 35 cm s^{-1}; fused silica dimethylpolysiloxane DB-1 60 m length, 0.25 mm i.d. and 0.25 μm film thickness; temperature programming from 50 to 238°C (8 min hold) increasing at 4°C min^{-1}; injector temperature 220°C, detector temperature 260°C.

GC/MS analyses

For the analysis of commercial essential oils of parsley, celery and dill, GC/MS (Hewlett Packard: HP 5890) conditions were as follows: inlet – split 1:25, carrier gas – helium 1 ml min^{-1}, columns-fused silica RSL-150, 40 m, i.d. = 0.32 mm, film thickness 0.2 μm; DB-5, 40 m, i.d. = 0.30 mm; temperature programming 35–220°C rising at 3°C/min, 220°C isothermal (20 min); detector – quadrupole type MS, no. 5970 series: MSD, temperature 250°C, injection 1 μl.

GC/MS analyses of caraway essential oils were performed on a HP 5890 with a 5970 series MSD in the EI ionisation mode at 70eV, and the following GC parameters: split inlet 1:10; helium as carrier gas at a flow rate of 2 ml min^{-1}; fused silica (50% phenyl) methylpolysiloxane DB-17 column, 30 m length, 0.25mm i.d., 0.25 μm film thickness, 50–250°C increasing at 3°C min^{-1}.

The identification of essential oil constituents was based on comparison of their spectra with reference spectra recorded under similar conditions with the same instrument, and with published spectra. Retention times expressed as Kovats indices were also used for identification (homologous series of C_6–C_{18} as standards). All GC analyses were replicated 4–5 times.

RESULTS AND DISCUSSION

Parsley

The list of the compounds identified in parsley essential oil and their relative amounts expressed as a percentage of the total area of the detected GC peaks are presented in Table 2.1. It is obvious that the application of two different columns enables us to show some additional data, especially for the compounds present in minor amounts. Thus, terpinolene, trans-β-ocimene and 1,3,8-*p*-menthatriene were detected only on the DB-5 column, and cuminaldehyde only on the RSL-150 column. The amounts of most compounds were very close for both columns. An exception was the cryptone peak, where the percentage area

almost four times larger when recorded on the DB-5 column than on the RSL-150 column. Compounds present in large quantities, such as β-phellandrene and limonene, were not separated on the DB-5 column, but their percentage amount was close to the sum obtained on the RSL-150 column.

Table 2.1. Chemical composition of parsley (*Petroselinum sativum* Hoffm.) essential oil.

Compound	Percentage composition		Retention Indexes (RI)
	RSL-150 column	DB-5 column	RSL-150
α-pinene	6.2	6.3	925
sabinene	0.3	0.2	–
β-pinene	3.6	3.0	967
myrcene	11.4	10.9	987
α-phellandrene	0.3	0.2	992
p-cymene	1.9	2.0	1006
β-phellandrene	21.1	–	1016
limonene	10.6	28.3	1019
terpinolene	nd	0.4	1040
trans-β-ocimene	nd	0.1	1048
α,p-dimethylstyrene	10.8	10.7	1085
1,3,8-p-menthatriene	nd	0.1	–
cryptone (4-isopropyl-cyclohex-2-enone)	0.5	1.9	1176
p-cymen-8-ol	0.7	1.1	1183
cuminaldehyde	0.3	nd	1232
β-caryophyllene	1.4	1.2	1411
β-selinene	0.8	0.7	1477
myristicin	20.4	17.9	1574
β-sesquiphellandrene	0.6	0.6	–
apiol	7.4	6.3	1679
TOTAL	**98.3**	**92**	

nd – not detected.

Comparison of the percentage composition of analysed parsley essential oil with published data shows that the amounts of the constituents are in the same order. For instance, Simon and Quinn (1988) characterised the following chemotypes of parsley according to the major constituent in quantity (as a percentage of the leaves' essential oil): 1,3,8-*p*-menthatriene (68%); myristicin (60%); β-phellandrene (33%), apiol (22%); myrcene (16%); terpinolene and 1-methyl-isopropenylbenzene (13%). An exception is 1,3,8-*p*-menthatriene, the amount of which was very low in our essential oil (0.07%). This compound is considered to be one of the key constituents of parsley-like aroma (Masanetz and Grosch, 1998; Lopez et al., 1999). The reason for the very small amount of 1,3,8-*p*-menthatriene could be either the specific chemotype of parsley or chemical changes during distillation and storage of the essential oil. Nitz et al. (1989) found that the decrease of the amount of 1,3,8-*p*-menthatriene (I) in parsley was accompanied by the increase of 1-methyl-4-(methylethenyl)-2,3-dioxabicyclo /2.2.2/-oct-5-ene (II) and 4-methyl-7-(methylethenyl)-3,8-dioxatricyclo /5.1.0/ octane (III).
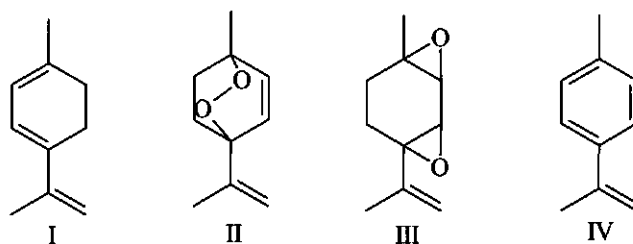
The similarity of the mass spectra of compounds I and III and the difference of 32 mass units between the molecular ions show that an addition of oxygen molecules to the menthatriene skeleton took place. The corresponding mass spectra of the essential oils analysed were not recorded. However, a considerable amount (10%) of α,p-dimethylstyrene (IV) was found, the structure of which is close to that of compound (II). Previously, it was reported as the major constituent of parsley leaves (Kasting et al., 1972).

The essential oil distilled from curly parsley leaves, which were obtained from a local market in Ghent, Belgium, contained 2% of 1,3,8-*p*-menthatriene. This sample was called "Myristicin" chemotype parsley, because it contained 69% of this component.

Celery

The compounds identified in celery essential oil and their relative amounts in percentageterms are presented in Table 2.2. Fourteen compounds were positively identified in celery essential oil, comprising 92% of the total area of peaks detected by GC. The main constituent in quantity, limonene (63%), was reported to be the major compound in celery (72.16%) by Cu et al. (1989) and by Macleod and Ames (1989) (35%). However, among 165 components characterised in celery, Wilson (1969) reported β-selinene and phthalides as the compounds possessing a characteristic celery aroma. β-Selinene (2%) and three phthalides (9%) were identified in the present study. The major phthalide was 3-*n*-butylphthalide, constituting 5.7%.

Table 2.2. Chemical composition of celery (*Apium graveolens*) essential oil.

Compound	Percentage composition	Retention Indexes (RI)
α-pinene	0.6	925
β-pinene	0.6	967
myrcene	12.2	987
p-cymene	1.9	1006
limonene	63.2	1019
cis-β-ocimene	0.5	1025
menthol	1.0	1160
carvone	0.7	1211
β-caryophyllene	0.2	1411
β-selinene	2.3	1477
β-caryophyllene oxide	0.3	1556
3-*n*-butylphthalide	5.7	1605
sedanenolide	0.4	1671
trans-neocnidilide	2.6	1678
TOTAL	**92.2**	

Dill

The list of volatile compounds identified in dill essential oil and their relative amounts expressed as a percentage from the total area of the detected GC peaks is presented in Table 2.3. Twelve compounds were positively identified in dill essential oil comprising 98.5% of the total detected GC/MS peaks area. The main constituents in quantity are the following: limonene (26%), α-phellandrene (23%), 3,6-dimethyl-2,3,3a,4,5,7a-hexahydrobenzofuran (dill ether) (21%), D-carvone (14%), *p*-cymene (5%), β-phellandrene (4%). These six compounds together comprised 98% of all identified compounds.

Table 2.3. Chemical composition of dill (*Anethum graveolens L.*) essential oil.

Compound	Percentage	Retention Index (RI)
α-thujene	0.2	
α-pinene	1.1	925
sabinene	0.1	
β-pinene	0.09	967
myrcene	0.6	987
α-phellandrene	23.1	992
p-cymene	5.5	1006
β-phellandrene	4.5	1016
limonene	26.6	1019
3,6-dimethyl-2,3,3a,4,5,7a-hexahydrobenzofuran (dill ether)	20.6	1165
D-carvone	14.2	1211
β-caryophyllene	0.08	1411
TOTAL	**96.7**	

The comparison of percentage composition with published data shows that the amounts of the major constituents were at the same order. According to the report of Badoc and Lamarti (1991) on three chemotypes of dill, the herb in this study analysed could be described as chemotype II (dillapiole), which is distinguished by a considerable amount of limonene, D-carvone, dill ether (dillapiole) and the absence of myristicin.

It is worth pointing out the high amount of dill ether (21%), which is considered to be a character impact compound, and is responsible for the major sensory properties of dill herb (Badoc and Lamarti, 1991). Mass spectra and the formula of this compound are presented in Fig. 2.1, its major m/z peaks were as follows: 137 (100), 69 (48), 109 (35), 41 (34), 79 (23), 55 (21), 91 (18). Göckeritz et al. (1979) were the first who identified this compound in dill herb. Furthermore, Schreier et al. (1981) consider this compound to be one of the major components of dill herb.
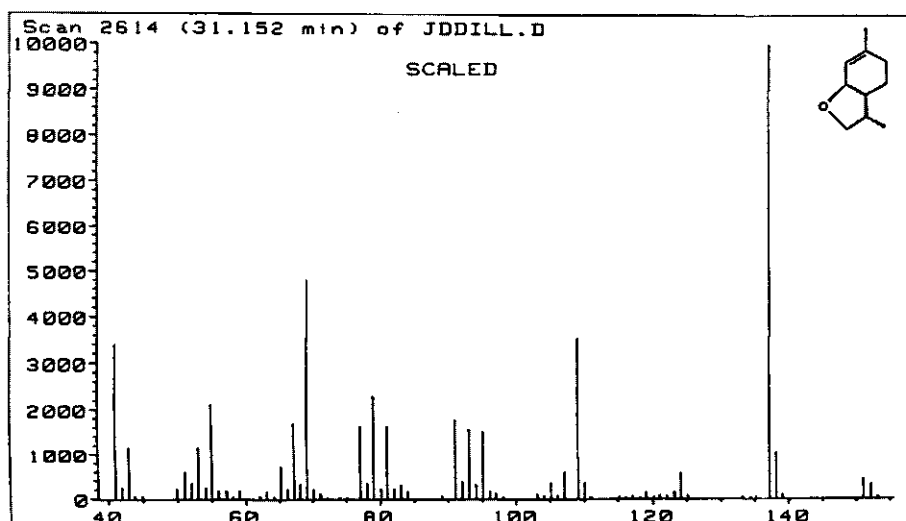

Fig. 2.1. Mass spectra and formula of 3,6-dimethyl-2,3,3a,4,5,7a-hexahydrobenzofuran (dill ether).

Caraway

General characterisation of some commercial and experimental caraway seed samples is presented in Table 2.4.

The yield of commercial caraway fruit samples presented in Table 2.4 varied from 984 to 1487 kg ha^{-1}. The content of essential oil in those samples was from 1.9 to 3.2 ml 100 g^{-1} and therefore the differences in the yield of essential oil per 1 ha was even greater, from only 20.7 to 46.4 dm^3 ha^{-1}. Very low yields from the samples of 1994 could be explained by the severe

draught in this year, which damaged the crops considerably. The differences in the yield of fruits, essential oil contents and consequently in the yield of essential oil per ha between different caraway cultivars were less considerable. Exceptionally good yields of caraway fruits (cultivar type Rekord) rich in essential oil were obtained during the fertilisation tests in Klausuciai. There is an evident increase in the yield gained by increasing the amount of nitrogen fertilisers applied. While the effect of nitrogen fertilisers on the essential oil content in the fruits was not very significant, the yield of oil per ha was considerably increased by using fertilisers, i.e. from 74.9 (N=0) to 106.4 dm^3 ha^{-1} (N=75).

Table 2.4. Characterisation of different caraway seed samples.

Caraway sample	Weight of 1000 seeds (g)	Yield of seeds (kg ha^{-1})	Oil content (cm^3 100 g^{-1})	Yield of oil (dm^3 ha^{-1})
Gintaras[1]	3.4	1093.8	4.0	43.8
Rekord[1]	3.4	875.0	4.1	35.7
Chmelnickij[1]	3.1	877.7	3.8	33.4
Prochana[1]	3.2	1000.3	4.0	40.3
Rekord[2] (N=0)*	3.2	1857.6	4.0	74.9
Rekord[2] (N=30)	3.5	1961.3	4.0	78.5
Rekord[2] (N=45)	3.4	2149.7	3.9	83.4
Rekord[2] (N=60)	3.3	2255.1	4.3	97.0
Rekord[2] (N=75)	3.2	2673.3	4.0	106.4
Skėmiai-1' 1994	3.1	984.0	2.1	20.7
Skėmiai-2' 1994	3.4	1312.0	2.3	30.2
Skėmiai 1995	3.4	1487.0	2.5	37.2
Meskuiciai 1994	3.1	1102.0	1.9	20.9
Panevėzys 1995	3.6	1450.0	3.2	46.4
Vilkija 1995	3.2	1021.0	2.7	27.6

Notes: [1]Experiments were performed at the experimental station of the Lithuanian Agricultural University, Kaunas region.
[2]Experiments were performed in Klausuciai, Jurbarkas region.
*The fertilisation of the other caraways was 30 kg ha^{-1} of nitrogen. Fertilisers: $NaNO_3$.
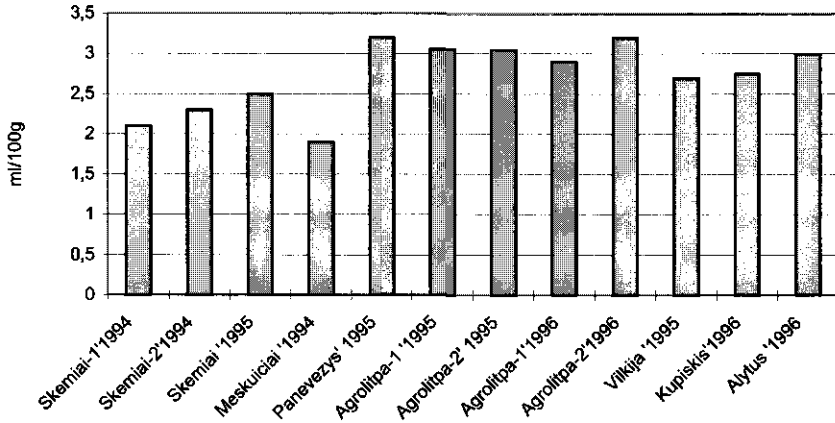
Fig. 2.2. Essential oil content in caraway seeds from various commercial suppliers

Considerably higher yields of caraway fruits obtained in the fertilisation tests as compared
with different cultivars grown in the experimental field of Lithuanian Agricultural University
(Kaunas region) can be explained by the differences in cultivation area: the conditions were
much more favourable in Klausuciai, Jurbarkas region, than at the experimental station of the
Lithuanian Agricultural University, Kaunas region. Bouwmeester et al. (1995b) discovered a
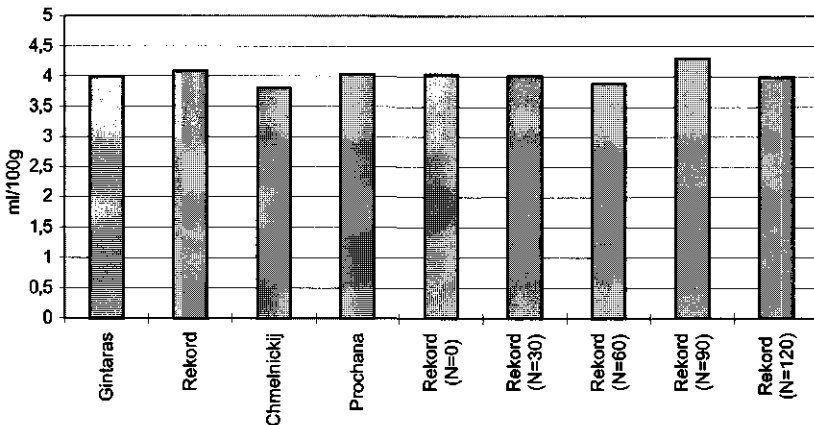close linear relationship between light intensity during caraway flowering and seed yield.

Fig. 2.3. Essential oil content in caraway cultivars (bars 1–4) and influence of
fertilisers content [kg ha^{-1}] on the yield of Rekord cultivar (bars 5–9).

Total essential oil content in commercial caraway samples varied from 1.9 to 3.3 ml 100 g^{-1} (Fig. 2.2). The differences in the total oil content between the four cultivars analysed were insignificant (Fig. 2.3: bars 1–4). Total essential oil content in caraway samples depending on the used amount of nitrogen fertilisers varied from 3.9 to 4.3 ml 100 g^{-1}. However, it is difficult to define clearly the effect of fertilisers on the oil content from the data obtained in this study (Fig. 2.3: bars 5–9).

Nine constituents were identified in the caraway oil samples: α-pinene, sabinene, myrcene, limonene, *cis*- and *trans*-dihydrocarvone, carvone, dihydrocarveol and β-caryophyllene. Carvone was the major constituent in all commercial caraway samples (53–63%) and in all four different cultivars (51–55%); limonene constituted from 36 to 45%, and from 41 to 44%, respectively (Figs 2.4 and 2.5).
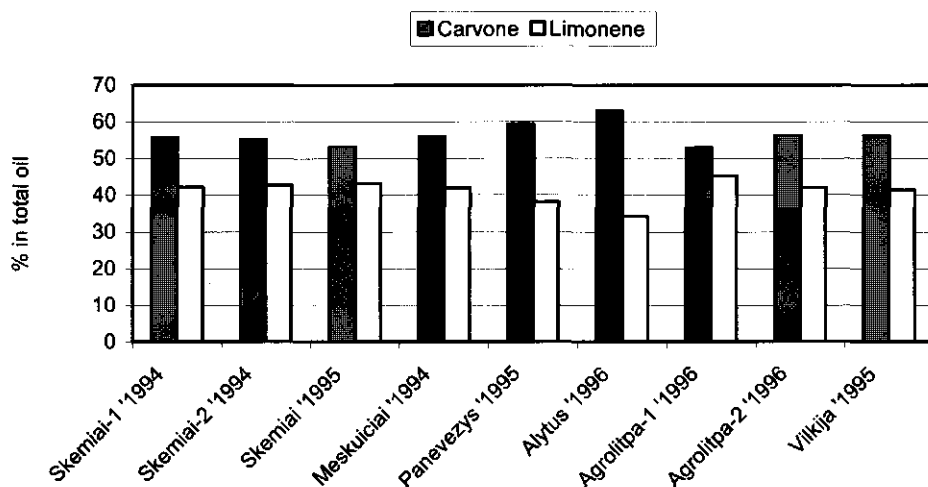
Fig. 2.4. Carvone and limonene content in caraway essential oils from various commercial suppliers.

The content of carvone and limonene in the caraway samples from the fields with different fertilisation was quite similar in all samples and varied from 47 to 53% and from 45 to 52% respectively. The concentration of limonene in some cases exceeded that of carvone, e.g. when fertilisation 120 kg ha^{-1} was used (Fig. 2.5).
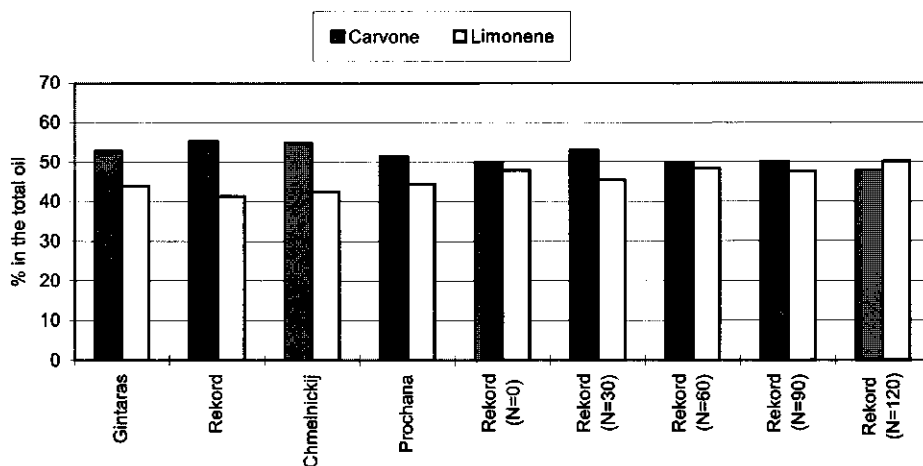
Fig. 2.5. Carvone and limonene content in the essential oil from different caraway cultivars and effect of fertilisers [kg ha^{-1}] on carvone and limonene content.

CONCLUSIONS

Chemical composition of essential oils of *Umbelliferae* plants (parsley, celery, dill and caraway) grown in Lithuania shows that the amount of the major constituents are in the same order as published data. β-Phellandrene (21%), myristicin (20%) and myrcene (11%) were found to be the most abundant constituents of parsley essential oil; limonene (63%) and myrcene (12%) were the major volatiles in celery essential oil. Aroma composition of dill herb was distinguished by a considerable amount of limonene (27%), D-carvone (14%), dill ether (21%) and the absence of myristicin, which indicates that the locally grown dill herb can be ascribed to chemotype II (dillapiole). In this way, the essential oils produced by company "Meta" are competitive products which can successfully replace imported oils.

The yield of caraway seeds cultivated in Lithuania and essential oil content in them varied in a rather large range, depending on different factors (cultivation site, cultivar, rate of fertilisation used, and climatic conditions). Carvone and limonene were the major constituents. The content of carvone was over 50%in almost all the samples analysed, which corresponds well with the requirements of the ISO. These results imply a clear need to optimise caraway cultivation parameters in order to obtain the highest yields of high quality essential oil. Understanding the relationship between agronomic factors and oil quality can make the

difference between a good yield of a high quality oil and a poor yield of an inferior oil and to produce reliable management guidelines for commercial growers.

REFERENCES

Analytical Methods Committee. Application of gas-liquid chromatography to the analysis of essential oils. Part XIV. Monograph for five essential oils. *Analyst* **1988**, 113, 1125–1136.

Appendino, G.; Jakupovic, J.; Bossio, E. Structural revision of the parsley sesquiterpenes crispanone and crispane. *Phytochemistry* **1998**, 49, 1719–1722.

Arganosa, G.C.; Sosulski, F.W.; Slinkard, A.E. Seed yields and essential oils of annual and biennial caraway (*Carum carvi* L.) grown in Western Canada. *J. Herbs Spices & Med. Plants* **1998**, 6, 9–17.

Badoc, A.; Lamarti, A.A. Chemotaxonomic evaluation of *Anethum graveolens* L. (Dill) of various origins. *J. Essent. Oil. Res.* **1991**, 3, 269–278.

Bartschat, D.; Beck, T.; Mosandl, A. Stereoisomeric flavor compounds. 79. Simultaneous enantioselective analysis of 3-butylphthalide and 3-butylhexahydrophthalide stereoisomers in celery, celeriac and fennel. *J. Agric. Food Chem.* **1997**, 45, 4554–4557.

Baslas, B.K.; Baslas, R.K. Aromen, Körperpflegem. **1972**, 22, 155.

Baysal, T.; Starmans, D.A.J. Supercritical CO_2 (SC-CO_2) extraction of carvone and limonene from caraway seed. *J. Supercrit. Fluids* **1999**, 14, 225–234.

Bjeldanes, L.F., Kim I-S. Phthalide components of Celery essential oil. *J. Org. Chem.* **1977**, 42, 2333–2335.

Bouwmeester, H.J.; Kuijpers, A.M. Relationship between assimilate supply and essential oil accumulation in annual and biennial caraway (*Carum carvi* L.). *J. Essent. Oil. Res.* **1993**, 5, 143–152.

Bouwmeester, H.J.; Smid, H.G. Seed yield in caraway (*Carum carvi*). 1. Role of pollination. *J. Agric. Sci.* **1995**, 124, 235–244.

Bouwmeester, H.J.; Davies, J.A.R.; Toxopeus, H. Enantiomeric composition of carvone, limonene and carveols in seeds of dill and annual and biennial caraway varieties. *J. Agric. Food Chem.* **1995a**, 43, 3057–3064.

Bouwmeester, H.J.; Smid, H.G.; Loman, E. Seed yield in caraway (*Carum carvi*). 1. Role of assimilate availability. *J. Agric. Sci.* **1995b**, 124, 245–251.

Bouwmeester, H.J.; Davies, J.A.R., Smid, H.G.; Welten, R.S.A. Physiological limitations to carvone yield in caraway (*Carum carvi* L.). *Ind. Crops Prod.* **1995c**, 4, 39-51.

Bouwmeester, H.J.; Gershenzon, J.; Konings, M.C.J.M.; Croteau, R. Biosynthesis of the monoterpenes and carvone in the fruit of caraway. *Plant Physiol.* **1998**, 117, 901–912.

Bouwmeester, H.J.; Konings, M.C.J.M.; Gershenzon, J.; Karp, F.; Croteu, R. Cytochrome P-450 dependent (+) – limonene-6-hydroxylation in fruits of caraway (*Carum carvi*). *Phytochemistry* **1999**, 50, 243–248.

British Pharmacopoiea. Vol. 1, HMSO: London, 1988.

Brunke, E.J.; Hammerschmidt, F.I.; Koester, F.H.; Mair, P. Constituents of Dill (*Anethum graveolens* L.) with sensory importance. *J. Essent. Oil. Res.* **1991**, 3, 257–267.

Chalcat, J.C.; Garry, R.Ph.; Michet, A.; Gorunovic, M. Huiles essentielles de *Carum carvi* de Yougoslavie. *Rivista Ital. EPOS* **1991**, Special Issue, 466–470.

Cu, J.; Zhang, Z.; Pu, F.; Perineau, F.; Delmas, M.; Gaset, A. GC/MS and GC/FTIR analysis of the essential oil of celery seed. *J. Essent. Oil Res.* **1989**, 2 (1), 1–5.

El-Wakeil, F.; Morsi, M.K.S.; Farag, R.S.; Shihata, A.A.; Badei, A.Z.N.A. Effects of various storage conditions on the quality of some spice essential oils. *Seifen Öle Fette Wachse* **1986**, 112, 348–353.

Evers, A.M.; Ketoja, E.; Hagg, M.; Plaami, S.; Hakkinen, U.; Pessala, R. Decreased nitrogen rates and irrigation effect on celery yield and internal quality. *Plant Foods for Human Nutr.* **1997**, 51, 173–186.

Formacek, V.; Kubeczka, K.H.; Essential Oils Analysis by Capillary Gas Chromatography and Carbon-13 NMR Spectroscopy. John Wiley & Sons, Chichester, 1982.

Göckeritz, D.; Poggendorf, A.; Schmidt, W.; Schubert, D.; Pohloudek-Fabini, R. Detection of 3,6-dimethyl-2,3,3a,4,5,7a-hexahydrobenzofuran in the essential oil dill. *Pharmazie* **1979**, 34, 846–847.

Haelvae, S.; Craker, L.E.; Simon, J.E.; Charles, D.J. Light levels, growth and essential oil in dill (*Anethum graveolens* L.). *J. Herbs Spices & Med. Plants* **1992a**, 1, 47–58.

Haelvae, S.; Craker, L.E.; Simon, J.E.; Charles, D.J. Light quality, growth, and essential oil in dill (*Anethum graveolens* L.). *J. Herbs Spices & Med. Plants* **1992b**, 1, 59–69.

Haelvae, S.; Craker, L.E.; Simon, J.E.; Charles, D.J. Growth and essential oil in dill, *Anethum graveolens* L., in response to temperature and photoperiod. *J. Herbs Spices & Med. Plants* **1993**, 1, 47–56.

Hälvä, S.; Hirvi, T.; Mäkinen, S.; Honkanen, E. Yield and glucosinolate of mustard seeds and volatile oils of caraway seeds and coriander fruit. II. Yield and volatile oils of caraway seeds (*Carum carvi* L.). *J. Agric. Sci. Finland* **1986**, 58, 163–167.

Halva, S.; Huopalahti, R.; Franz, R.; Makinen, S. Herb yield and essential oil of dill (*Anethum graveolens* L.) at different locations. *J. Agric. Sci. Finland* **1988**, 60, 93–100.

Heath H.B. Source book of flavours.AVI Publishing Company Inc USA,1981.

Hungarian Standard MSZ 9265, Caraway Essential Oil. 1981, 3 pp

Huopalahti, R.; Linko, R.R. Composition and content of aroma compound in Dill, *Anethum graveolens* L., at three different growth stages. *J. Agric. Food Chem.* **1983**, 31, 331–333.

Hussien, M.S. Response of growth, yield and essential oil of coriander and dill to different nitrogen sources. *Egyptian J. Horticulture* **1995**, 22, 1–10.

ISO Oil of Caraway (*Carum carvi* L.). ISO-8896-87, 1987.

Jirovetz, L.; Buchbauer, G.; Nikiforov, A. Comparative analysis of different dill herb and dill seed oil constituents by means of GC/FID and GC/MS. Vergleichende Inhaltsstoffanalyse verschiedener Dillkraut- und Dillsamenoele mittels GC/FID und GC/MS. *Ernaehrung* **1994**, 18, 534–536.

Kallio, H.; Kerrola, K.; Alhonmäki, P. Carvone and limonene in caraway fruits (*Carum carvi* L.) analyzed by supercritical carbon dioxide extraction-gas chromatography. *J. Agric. Food. Chem.* **1994**, 42, 2478–2485.

Kasting, R.; Andersson, J.; von Sydow, E. Volatile constituents in leaves of parsley. *Phytochemistry* **1972**, 11, 2277–2282.

Lawrence, B.M. New trends in essential oils. *Perfum. Flavor.* **1980a**, 5, 6–16.

Lawrence, B.M. Dill oil. In: Progress in essential oils. *Perfum. Flavor.* **1980b**, 5 , 49–50.

Lopez, M.G.; Sanchez-Mendoza, I.R.; Ochoa-Alejo, N. Comparative study of volatile components and fatty acids of plants and in vitro cultures of parsley (*Petroselinum crispum* (Mill) Nym ex Hill). *J. Agric. Food Chem.* **1999**, 47, 3292–3296.

Macleod, G.; Ames, J.M. Volatile components of Celery and Celeriac. *Phytochemistry* **1989**, 28, 1817–1824.

MacLeod, A.J.; Snyder, C.H.; Subramanian, G. Volatile aroma constituents of parsley leaves. *Phytochemistry* **1985**, 24, 2623-2627.

Masanetz, C.; Grosch, W. Key odorants of parsley leaves (*Petroselinum crispum* [Mill.] ssp. crispum) by odour – activity values. *Flavour Fragrance J.* **1998**, 13, 115–124.

Nitz, S; Kollmannsberger, H; Spraul, M.H; Drawert, F. Oxygenated derivatives of menthatriene in parsley leaves. *Phytochemistry* **1989**, 28, 3051–3054.

Pino, J.A.; Roncal, E.; Rosado, A.; Goire, I. Herb oil of dill (*Anethum graveolens* L.) grown in Cuba. *J. Essent. Oil Res.* **1995**, 7, 219–220.

Puschmann, G.; Stephani, V.; Fritz, D. Untersuchungen zur Variabilität von Kümmel (*Carum carvi* L.). *Gartenbauwissenschaft* **1992**, 57, 275–277.

Richard, H.; Noleau, I.; Giampaoli, P. Techniques d'analyse des épices et aromates. In: *Epices et Aromates;* Richard, H. (Coordinateur), Tec & Doc-Lavoisier Apria, 1992; pp 192–213.

Schreier, P.; Drawert, F.; Heindze, I. Quantitative composition of natural and technologically modified plant aromas. IX. Freeze concentration of an extract from fresh dill herb. *Z. Lebensm. Unters. Forsch.* **1981**, 172, 257–263.

Simon, J.E.; Quinn, J. Characterization of essential oil of parsley. *J. Agric. Food Chem.* **1988**, Vol. 36, 467–472.

Uhlig, J.W.; Chang, A.; Jen, J.J. Effect of phthalides on Celery flavour. *J. Food Sci.* **1987**, 52 (3), 658–660.

Van Wassenhove, F.; Dirinick, P.; Schamp, N. Analysis of the key components of Celery by two dimensional gas chromatography. In: *Bioflavour 87;* Schreier P., ed.; Analysis-Biochemistry-Biotechnology. Berlin: Walter de Gruyter, 1988; pp 137–144.

Wilson, C.W. Terpene and sesquiterpene hydrocarbons in the essential oil from fresh celery. *J. Food Sci.* **1969**, 34, 521.

Zawirska-Wojtasiak, R.; Wasowicz, E.; Jelen, H.; Rudzinska, M.; Kaminski, E.; Blazczak, P. Aroma characteristics of dill seeds varieties grown in Poland. *Polish J. Food Nutr. Sci.* **1998**, 7, 181–192.

INFLUENCE OF HARVESTING TIME ON THE COMPOSITION OF VOLATILE COMPONENTS IN DIFFERENT ANATOMICAL PARTS OF LOVAGE (*LEVISTICUM OFFICINALE* KOCH.)

This chapter is based on the following article:

ABSTRACT

The essential oils from different botanical parts (leaves, stems, flowers and seeds) of lovage (*Levisticum officinale* Koch.) were analysed at various phases of plant growth. The seasonal changes in leaves were less considerable than in the stems. Seeds and flowers possessed the highest yield of oil. α-Terpinyl acetate was found to be dominating compound in leaves and stems (up to 70%), β-phellandrene in seeds and flowers (61.5 % and 40.8 % respectively); Z-ligustilide was confirmed as a major lovage phthalide constituting from 4.4% to 11.7% in leaves and from 4.8% to 13.8% in the stems essential oils depending on the harvesting time.

INTRODUCTION

Lovage (*Levisticum officinale* Koch.) is a tall perennial aromatic plant, cultivated in numerous European countries. The essential oils from leaves, seeds and roots of lovage are used in food, beverage, perfumery, and tobacco industry (Cu et al., 1993). The root of lovage has also been known for centuries as a medicine possessing carminative and spasmolytic activity (Segebrecht and Scilcher, 1989).

The composition of volatile compounds of lovage has been the subject of numerous investigations (Gijbels et al., 1982; De Pooter et al., 1985; Toulemonde et al., 1987; Toulemonde and Noleau, 1988; Segebrecht and Scilcher, 1989; Cu et al., 1990; Stahl-Biskup and Wichtmann, 1991; Blank and Schieberle, 1993; Venskutonis, 1995; Bylaite et al., 1996). Different anatomical parts of lovage have been analysed, however, most of the studies were focused on the root. Their specific flavour is characterised as medium aromatic (Heath and Reineccius, 1986) or as strong impact warmly aromatic with sweet, yeasty, musky, lemon-like, celery-like notes (Heath, 1981). The phthalides are believed to play a major role in the aroma of lovage. Seven phthalides have been identified in lovage roots constituting from 22% to 71% (Toulemonde and Noleau, 1988; Cu et al., 1990) in the total essential oil. Different isolation procedures (dynamic headspace, distillation, and extraction with different solvents) were used to obtain lovage aroma concentrates and the content of phthalides in them was highly dependent on the method applied. However, only a few studies on the assessment of odour active components of lovage were performed in recent years (Blank and Schieberle, 1993; Bylaite et al., 1996) and none of them demonstrated the significant contribution of

phthalides to the aroma of lovage. On the contrary, the investigation of celery aroma compounds revealed a significant correlation between sensory evaluation of flavour scores with the individual and total phthalide contents (Uhlig et al., 1987; MacLeod and Ames, 1989).

Toulemonde and Noleau (1988) performed a detailed study of lovage leaf, seed and root essential oils as well as root and leaf solvent extracts in 1988, and identified 191 compounds. Quantitative analysis of seed and root essential oil showed that β-phellandrene was a major component in the seed essential oil (63%) whilst *cis*-3-*n*-butylidene-4,5-dihydrophthalide was a dominating constituent in the root oil (67%). It should be noted that the number of detected compounds significantly depended on the method of isolation, e. g., 116 compounds were detected in the leaves by using simultaneous distillation-extraction in the Likens-Nickerson apparatus, whereas only 31 constituents were found in dichloromethane extract. De Pooter et al. (1985) also performed comparison of different isolation procedures and their effects on aroma composition of lovage. They found that the headspace of fresh lovage constituted almost exclusively of mono- and sesquiterpenes, while phthalides appeared among volatiles only when hydrodistillation was used.

The variation of aroma composition depends on different factors, such as the isolation procedure, the botanical part of the plant, harvesting time and state of maturity. A survey of the literature reveals that a detailed investigations of the variations of yield and changes of aroma compounds in different botanical parts of lovage during the growth period have not been the subject of any detailed study. An exception was the study by Stahl-Biskup and Wichtmann (1991), in which the composition of the essential oil from the lovage roots in relation to the development of their oil duct systems was analysed and phthalides were found in considerable amounts only after many secondary oil ducts had been developed.

Thus it was considered that the studies of the seasonal variations in the composition of volatiles in different botanical parts of lovage cultivated in Lithuania would be of some interest.

MATERIALS AND METHODS

Plant material and isolation of volatile compounds

Plant material was collected in the experimental garden of Lithuanian Institute of Horticulture in 1995. The whole herb was harvested every second week, total in 8 times from the middle of May untill the end of September. The plants were sorted by botanical part (leaves, stems, flowers, seeds, and roots) depending on the growth period and air-dried at 30° in the laboratory oven with active ventilation. Volatiles were isolated by hydrodistillation in a Clevenger type apparatus during 2 hr. Triplicate analysis was carried out for every sample. Simultaneously the content of moisture in air-dried herb was determined by distilling 5 g of the sample with toluene for 1.5 hr (AOAC, 1995). The yields of oil were presented in % (w/w) of dry wt.

Identification and quantification of volatile compounds

The essential oils were diluted with Et_2O (1% v/v) and analysed on a dual column Carlo Erba Vega 6000 gas chromatograph with FID heated at 260°C. Four GC analyses were carried out for each essential oil. Two different polarity fused silica capillary columns were used: DB-1 (dimethylpolysiloxane) and DB-WAX (polyethylene glycol) both 60 m length, 0.25 mm i. d., 0.25 μm film thickness. The oven temperature was programmed from 50°C (5 min hold) to 250°C at a rate of $3°C \ min^{-1}$ with a final hold of 10 min. Helium was used as a carrier gas with a flow rate of $2 \ ml \ min^{-1}$. Injector temperature was set up at 230°C.

Gas chromatography-mass spectrometry analyses were performed on a combined GC-MS (Varian 3400, Finnigan MAT 95) instrument with a mass selective detector by using BP-5 (5% phenyl-methylpolysiloxane) capillary column (25 m length, 0.25 mm i. d., 0.25μm film thickness); GC oven temperature was programmed from 60°C to 260°C at the increase of $3°C \ min^{-1}$. Mass spectra were obtained with 70 eV electron impact ionisation and scanned from m/z=24 to 300 at $0.5 \ s \ dec^{-1}$.

Identification was based mainly on the comparison of retention indices (RI) and mass spectra (Adams, 1995). Also the results obtained were thoroughly compared with the published ones

and found to be in a good agreement. When available, pure chemicals were used as reference compounds. The content of the individual constituents was expressed as a peak area percent computed by GC system integrator, and also in arbitrary units, which were calculated by the following formulae:

$$C = \frac{Y \times I \times 1000}{100}$$

C - amount of individual component in arbitrary units (a.u.);

Y - yield of essential oil, % of dry wt;

I - percentage content of individual component in the essential oil.

An arbitrary unit represents absolute concentration of a particular compound in 1 kg of dry herb. An arbitrary unit is approximately equal to the $\mu g\ kg^{-1}$, and it could be converted into a $\mu g\ kg^{-1}$ by determining GC response factors for every identified constituent.

RESULTS AND DISCUSSION

Total essential oil content

The yields of lovage essential oil at different harvesting periods are given in Table 3.1. The highest amount of essential oil (2.70%) was determined in the seeds, which were formed in the middle of July. The concentration of volatile oil in leaves and stems varied from 1.01 to 1.35% and 0.53-1.16% respectively, whereas the flowers (July 7) contained 1.53% of oil.

It should be emphasised that lovage having a high concentration of oil in its stems is an exceptional plant as compared with many other aromatic herbs usually possessing only a negligible amount of volatiles in their stems (Venskutonis, 1995). However, an extremely low amount of oil was obtained by hydrodistillation from the roots (approx. 0.05%) and this finding is in disagreement with the results obtained by Maghami (1979) who reported the content of oil in lovage roots to range from 0.60-1%, in the seeds from 0.80-1.10%, and in the leaves from 0.05-0.15%. A much higher content of oil in lovage roots was also determined by Cu et al. (1990).

Table 3.1. Yields % (w/w of dry wt) of essential oils from different botanical parts of lovage at different harvesting periods

Growth phase and date of harvesting	Anatomical part				
	Leaves	Stems	Flowers	Seeds	Roots
1. Shoot up, May 15	1.14±0.04	0.53±0.04			
2. Growing, May 25	1.21±0.05	0.68±0.04			
3. Growing, June 9	1.35±0.04	0.97±0.04			
4. Formation of buds, June 16	1.12±0.06	1.16±0.06			
5. Beginning of flowering, June 28	1.01±0.04	0.65±0.04			
6. End of flowering, July 7	1.10±0.05	0.53±0.03	1.53±0.06		
7. Formation of seeds, July 19	1.09±0.04	0.73±0.03		2.7±0.05	
8. 2nd shoot up (newly grown plant), Sept 21	1.10±0.03	1.16±0.03			0.05±0.04

The content of essential oil in the leaves was almost constant during the whole period of plant vegetation with a peak (1.30%) just before the formation of buds (the phase of intensive growing, beginning of June). From a previous harvest in 1992 the yield of essential oil from the leaves of lovage on June 17 (beginning of blossom) and July 8 (full bloom) was significantly higher, 1.47 and 1.98% respectively, than the yield on 1995 most likely due to different climatic conditions, because the cultivars of the plant, growing and drying conditions were the same (Venskutonis, 1995).

The concentration of the essential oil in the stems was steadily increasing from 0.53% in May 15 (initial growing) to 1.16% in June 16 (buds formation), however, afterwards dropped again. In general, it is accepted that most of the herbs accumulate the highest amount of volatile oil during the anthesis period, however numerous studies show that every particular aromatic plant can possess its own peculiarities. For instance, the study of aroma compounds in dill by Huopalahti and Linko (1983) showed their highest value at the initial stage of growth, whilst the thyme was richest in essential oil during or immediately after the full bloom (Senatore, 1996).

Composition of essential oils

The yield of root essential oil (0.05%) was very low which makes it difficult to separate the oil from the distillate. Therefore, for further analyses only the oils from leaves, stems, seeds and flowers were used.
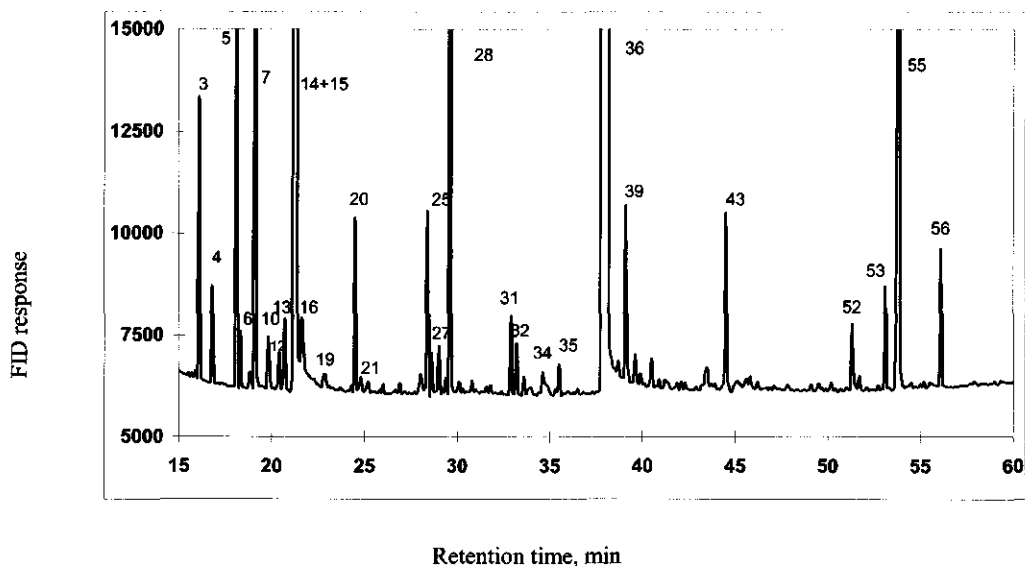
Fig. 3.1. Gas chromatogram of the essential oil of lovage leaves harvested in May 15. See Materials and Methods section for the GC conditions and Table 2 for the peak identifications

Fifty-eight compounds were identified in different parts of lovage and are listed in Table 3.2 in order of their elution from the DB-1 column. Most of them have been already reported in previous studies of the lovage (Toulemonde et al. 1987; Toulemonde and Noleau, 1988; Cu et al. 1993;) excepting for some minor tentatively identified constituents (No. 23, 24, 29, 30, 35, 37, 41, 45, 46, 47). The quantitative composition of the oils was almost similar in all samples except for some minor components, which were detected only in particular botanical parts. Although most of the identified constituents were present in the essential oils during all investigation seasons, a few minor ones were not detected at some harvesting periods. For further discussion trace compounds of lovage (<0.1 %) have not been considered.

Table 3.2 Identification data of volatile compounds in different botanical parts of lovage

No[1].	Constituent[2]	Leaves	Stems	Seeds	Flowers	Content, % (min-max)
1.	tricyclene	a,b	a,b	-	-	tr
2.	α-thujene	a,b,c	a,b,c	a,b,c	a,b,c	tr-0.11
3.	α-pinene	a,b,c	a,b,c	a,b,c	a,b,c	0.37-1.47
4.	camphene	a,b,c	a,b,c	a,b,c	a,b,c	tr-0.43
5.	sabinene	a,b,c	a,b,c	a,b,c	a,b,c	0.45-1.21
6.	β-pinene	a,b,c	a,b,c	a,b,c	a,b,c	tr-0.77
7.	myrcene	a,b,c	a,b,c	a,b,c	a,b,c	1.30-7.12
8.	2,3-dehydro-8-cineole	a,b	a,b	-	a,b	tr-0.17
9.	octanal	a,b	b	b	b	tr-0.21
10.	α-phellandrene	a,b,c	a,b,c	a,b,c	a,b,c	0.15-1.96
11.	Δ-3-carene	-	-	a,b	-	tr
12.	α-terpinene	a,b,c	a,b,c	a,b,c	a,b,c	tr-0.48
13.	*p*-cymene	a,b,c	a,b,c	a,b,c	a,b,c	tr-0.92
14.	β-phellandrene	a,b,c	a,b,c	a,b,c	a,b,c	10.85-61.50
15.	limonene	a,b,c	a,b,c	a,b,c	a,b,c	1.90-5.38
16.	β-ocimene (Z)	a,b,c	a,b,c	a,b,c	a,b,c	0.13-1.64
17.	phenyl acetaldehyde	a,b	-	-	-	tr
18.	β-ocimene (E)	a,b,c	a,b,c	a,b,c	a,b,c	tr-0.39
19.	γ-terpinene	a,b,c	a,b,c	a,b,c	a,b,c	0.16-1.87
20.	terpinolene	a,b,c	a,b,c	a,b,c	a,b,c	tr-1.72
21.	linalool	a,b,c	a,b,c	a,b,c	a,b,c	tr-0.23
22.	2-methylbutyl-2-methylbutanoate	a	a	-	-	tr
23.	isomer of compound no. 22	a	a	-	-	tr
24.	*cis-p*-menth-2-en-1-ol	a	-	-	-	tr
25.	pentylcyclohexadiene	a,b,c	a,b,c	a,b,c	a,b,c	0.21-0.95
26.	terpinen-4-ol	a,b,c	a,b,c	a,b,c	a,b,c	tr-0.23
27.	4-isopropyl cyclohex-2-en-1-one	a	a	a	a	tr-0.80
28.	α-terpineol	a,b,c	a,b,c	a,b,c	a,b,c	tr-1.50
29.	methyl salycilate	-	-	a,b	-	0.11
30.	carvone	a,b	-	-	a,b	tr-0.27
31.	geraniol	a,b,c	b,c	-	-	tr-0.22
32.	linalool acetate	b	a,b	-	-	tr

33.	phellandral	-	-	-	a	0.10-0.13
34.	bornyl acetate	a,b,c	a,b,c	a,b,c	a,b,c	0.11-0.61
35.	*cis*-dihydro-α-terpinyl acetate	b	b	b	b	tr-1.35
36.	α-terpinyl acetate	a,b,c	a,b,c	a,b,c	a,b,c	4.56-70
37.	bornyl propanoate	-	a	-	a	tr-0.81
38.	α-copaene	-	-	a	a	tr
39.	geranyl acetate	a,b,c	a,b,c	-	a,b,c	tr-1.52
40.	methyleugenol	-	-	a,b	a,b	0.10-0.20
41.	bornyl 2-methylpropanoate	-	a	-	-	tr
42.	β-farnesene	-	-	a,b	a,b	0.13-0.78
43.	germacrene D	a,b,c	a,b,c	a,b,c	a,b,c	tr-1
44.	β-selinene	a,b,c	a,b,c	a,b,c	a,b,c	tr
45.	germacrene A	a,b	a,b	a,b	a,b	tr-0.25
46.	bornyl 2-methylbutanoate	-	a	-	-	tr
47.	bornyl 3-methylbutanoate	a	a	-	-	tr
48.	δ-cadinene	a	-	a	a	tr
49.	germacrene B	a,b	-	a,b	a,b	tr
50.	α-cadinol	-	-	-	a	tr
51.	3-butylphthalide	a	-	-	a	tr
52.	3-butylidene-phthalide (Z)	a,b,c	a,b,c	a,b,c	a,b,c	tr-0.69
53.	3-butylidene-phthalide (E)	a,b,c	a,b,c	a,b,c	a,b,c	0.10-0.38
54.	sedanolide	a	a	-	-	tr
55.	ligustilide (Z)	a,b,c	a,b,c	a,b,c	a,b,c	4.44-16.01
56.	ligustilide (E)	a,b,c	a,b,c	a,b,c	a,b,c	0.13-0.47
57.	validene-4,5-dihydrophthalide	-	-	-	a	tr
58.	neophytadiene	-	a	-	-	tr

[1]peak numbers refer to Fig. 3.1

[2]constituents are listed in order of elution from DB-1 column

a - identification confirmed by mass spectra

b - identification confirmed on DB-1 column

c - identification confirmed on DB-Wax column

Percentage composition of the compounds exceeding 0.10% in the total essential oil is presented in Table 3.3.

Table 3.3 Percentage composition of essential oils from various anatomical parts of lovage at different growth phases (1-8 as in Table 3.1)

Component	RI (DB-1)	Leaves 1	2	3	4	5	6	7	8	Stems 1	2	3	4	5	6	7	8	Seeds 7	Flowers 6
α-thujene	924	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	0.11
α-pinene	928	0.37	0.58	0.46	0.58	0.70	0.60	0.62	0.77	1.25	1.16	1.08	1.01	0.76	1.13	1.06	1.15	1.47	1.01
camphene	940	0.10	0.17	tr	0.15	0.15	0.14	0.13	0.16	0.43	0.38	0.29	0.31	0.23	0.35	0.31	0.31	0.25	0.21
sabinene	962	0.52	0.69	0.56	0.78	0.82	1.07	1.01	1.04	1.21	0.76	0.73	0.70	0.45	0.69	0.81	0.78	0.54	1.00
β-pinene	967	tr	0.12	tr	0.12	0.17	0.14	0.15	0.20	0.77	0.57	0.21	0.16	0.22	0.37	0.38	0.54	0.35	0.31
2,3-dehydro-1,8cineole	975	tr	tr	tr	tr	tr	tr	tr	tr	0.17	tr	0.16	tr	tr	tr	tr	tr	nd	tr
myrcene	981	1.60	1.94	1.81	2.79	3.43	4.44	3.98	3.95	1.30	1.55	1.39	2.69	1.52	2.06	2.68	3.34	2.19	7.12
octanal	992	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	0.21	0.17
α-phellandrene	994	0.48	0.53	0.45	0.45	0.95	0.68	0.72	0.72	0.15	0.23	0.17	0.35	0.22	1.22	0.40	0.84	1.96	0.93
α-terpinene	1006	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	0.48	tr	tr
p-cymene	1008	tr	0.13	0.18	0.17	0.27	0.33	0.36	0.17	0.20	0.13	0.13	tr	0.13	0.17	0.16	0.16	0.59	0.92
β-phellandrene	1019	13.43	17.90	14.39	17.53	20.08	20.52	21.37	21.15	13.26	13.20	12.01	12.17	10.85	16.32	15.78	19.21	61.50	40.82
limonene	1020	2.35	2.46	1.90	2.07	4.65	2.27	2.22	2.05	2.54	2.45	2.31	2.04	2.20	5.38	2.52	4.24	3.16	2.25
β-ocimene(Z)	1025	0.62	1.14	0.77	0.49	1.64	0.18	0.98	1.01	0.13	0.57	0.59	0.26	1.04	0.13	1.28	0.88	0.54	2.50
β-ocimene (E)	1036	tr	tr	tr	tr	0.21	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	0.39	0.24
γ-terpinene	1047	0.23	0.37	0.52	0.39	1.20	1.56	1.58	1.57	0.26	0.21	0.19	0.16	0.20	0.83	0.38	0.70	0.39	1.87
terpinolene	1077	0.34	0.40	0.26	0.25	1.58	0.37	0.36	0.25	0.36	0.33	0.39	0.34	0.39	1.72	0.37	1.08	tr	0.11
linalool	1082	tr	tr	tr	tr	tr	tr	tr	tr	0.23	tr	tr	tr	tr	tr	tr	tr	0.11	0.22
pentylcyclohexadiene	1144	0.27	0.54	0.46	0.52	0.95	0.83	0.50	0.54	0.21	0.27	0.23	0.28	0.24	0.30	0.31	0.46	0.43	0.40
4-isopropyl cyclohex-2-en-1-one 1148		tr	tr	tr	tr	tr	tr	tr	tr	0.12	0.10	0.11	tr	0.16	tr	tr	tr	0.80	0.44
terpinen-4-ol	1155	tr	tr	0.11	tr	tr	tr	tr	tr	0.19	0.14	0.16	0.17	0.15	0.23	0.17	0.15	tr	0.14
α-terpineol	1165	1.01	1.50	1.12	1.04	1.02	0.69	0.88	0.88	1.22	1.07	1.43	1.07	1.48	1.00	1.01	0.86	tr	0.31
methyl salicylate	1169	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.11	nd
carvone	1220	tr	tr	tr	tr	tr	tr	tr	tr	nd	nd	nd	nd	nd	nd	nd	nd	0.10	0.27
geraniol	1232	0.12	0.12	0.18	0.16	0.13	0.12	0.14	0.11	tr	tr	tr	0.22	0.21	tr	tr	0.10	nd	nd

Compound	RI																		
phellandral	1237	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.10	0.15
bornyl acetate	1264	0.17	0.19	0.12	0.15	0.14	0.13	0.11	0.13	0.61	0.54	0.28	0.38	0.43	0.51	0.32	0.26	0.13	0.14
cis-dihydro-terpinyl acetate	1281	tr	tr	tr	tr	tr	tr	tr	tr	nd	nd	nd	nd	nd	nd	nd	nd	1.35	0.79
α-terpinyl acetate	1338	70.00	59.74	65.10	60.42	49.70	51.67	53.70	53.42	66.70	65.26	68.97	60.32	62.10	49.14	56.90	48.20	4.56	16.27
geranyl acetate	1358	0.83	0.67	0.54	0.39	0.45	0.41	0.46	0.69	1.37	1.52	1.28	1.03	1.35	1.43	1.30	1.01	nd	tr
bornyl propanoate	1362	nd	nd	nd	nd	nd	nd	nd	nd	0.13	0.81	0.58	tr	tr	0.71	tr	tr	nd	nd
methyleugenol	1379	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.20	0.10
β-farnesene	1441	nd	nd	Nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.78	0.13
germacrene D	1473	0.39	0.26	0.30	0.29	0.35	0.47	0.44	0.54	tr	tr	tr	tr	tr	tr	tr	tr	0.82	1.00
germacrene A	1501	tr	tr	tr	tr	tr	tr	tr	tr	0.24	0.20	0.14	0.23	0.23	0.25	0.20	0.21	tr	tr
bornyl 2-methylbutanoate	1488	nd	nd	nd	nd	nd	nd	nd	nd	0.35	0.29	0.20	0.18	0.15	0.22	0.16	0.12	nd	nd
germacrene B	1546	tr	tr	tr	tr	tr	tr	tr	tr	nd	nd	nd	nd	nd	nd	nd	nd	0.47	0.37
3-butyliden-phthalide (Z)	1631	tr	0.12	0.14	0.22	0.19	0.21	0.16	0.19	0.30	0.29	0.21	0.23	0.55	0.26	0.26	0.23	0.69	0.51
3-butyliden-phthalide (E)	1677	0.14	0.18	0.18	0.17	0.10	0.16	0.11	0.10	0.26	0.21	0.27	0.23	0.38	0.22	0.19	0.13	0.26	0.34
ligustilide (Z)	1697	4.44	7.60	7.51	7.85	6.49	11.70	8.39	9.73	4.80	6.35	5.13	12.90	13.85	7.72	10.56	11.24	14.18	16.01
ligustilide (E)	1754	0.36	0.39	0.23	0.16	0.14	0.26	0.21	0.17	0.13	0.19	0.30	0.36	0.47	0.18	0.29	0.30	0.23	0.27
TOTAL		97.77	97.74	97.29	96.56	95.51	98.95	98.58	99.54	97.64	97.62	97.86	96.78	99.20	91.41	96.74	95.83	98.86	97.47

tr = traces =<0.1%; nd - not detected

All identified constituents were divided into the three groups: major constituents (the mean content higher than 9%), compounds of low-medium concentration (constituting approximately 1-5%), and minor constituents (less than 1%). Three major components were common for all analysed lovage parts, namely, β-phellandrene, α-terpinyl acetate and *cis*-ligustilide. This finding is in agreement with the results published in the literature (Toulemonde and Noleau, 1988; Venskutonis, 1995). However the differences in the ratio and seasonal variations in the content of these components in separate parts of the plant were quite considerable (Table 3.2).

Major constituents of lovage

A monoterpene ester, α-terpinyl acetate, was the major constituent in the essential oils from stems and leaves at all harvesting periods: its peak constituted 48.20-68.97% and 49.70-70% respectively in the total GC peak area. The highest content of this compound (70%) was determined in the leaves collected during the first harvesting in May 15; in the stems it was also high at this vegetation phase and exceeded 65% in the period from May 15 till June 9. Some reduction in the percentage (Table 3.3) and absolute (Fig. 3.2) concentration of α-terpinyl acetate in the leaves and stems can be observed during the flowering period of the plants. In the flowers it constituted only 16.27% (July 7) but the smallest amount of α-terpinyl acetate was determined in the seeds 4.56%. These results are in agreement with published data by Toulemonde and Noleau (1988) who found essential oil from seeds containing 63.15% and 3.07% of β-phellandrene and α-terpinyl acetate respectively. The impact of α-terpinyl acetate on the aroma profile of lovage was not studied in the surveyed literature. The odour of this compound is characterised as "fresh bergamot-lavender" (Bauer et al., 1990), and it would be interesting to establish its role in the lovage aroma profile. Recently in a gas chromatography-sniffing panel technique only 3 out of 10 panellists detected α-terpinyl acetate in the headspace vapour concentrate from lovage stems and flowers by attributing floral, sweet odour tones to it (Bylaite et al., 1996).

The content of the major monoterpene hydrocarbon β-phellandrene varied from 13.43 to 21.37% of the total essential oil of leaves and from 10.85 to 19.21% in stems. It is interesting to notice that it increased during flowering both in leaves (5th harvesting, June 28) and stems (6th harvesting, July 7). During this phase the percentage content of α-terpinyl acetate

decreased. β-Phellandrene was also the major constituent in seeds (61.50%) and flowers (40.82%). It was described as an odour active compound having a grassy, chemical odour in our previous study of lovage (Bylaite et al., 1996).

Z-Ligustilide is known as a major lovage phthalide (Gijbels et al., 1982; Toulemonde and Noleau, 1988; Cu et al., 1990; Stahl-Biskup and Wichtmann, 1991; Venskutonis, 1995;) and it was found in this study as well. The percentage concentration of Z-ligustilide varied from 4.44% to 11.70% in the leaves and from 4.80 to 13.85% in the stem essential oil depending on the time of harvesting. Its content was slightly higher in the seeds (14.18%) and flowers (16.01%). Corresponding contents of Z-ligustilide were found in the leaves and stems of lovage from Lithuania harvested in 1992 (Venskutonis, 1995). It can be noticed that the content of Z-ligustilide (Table 3.3) was lower both in leaves and stems at the initial phase of growing.

Low-medium concentration constituents of lovage

The following compounds can be included in this group: α-pinene, sabinene, myrcene, limonene, *cis*-β-ocimene, γ-terpinene, α-terpineol, and geranyl acetate, of which limonene and myrcene were the most abundant components. Limonene ranged from 1.90 to 4.65% in the leaves and from 2.04 to 5.64% in the stems, myrcene from 1.60 to 4.44% in the leaves and from 1.30 to 3.34% in the stems. The highest content of myrcene was determined in the flowers - 7.12%. Although the changes in monoterpene hydrocarbon content at different harvesting period are of a somewhat "jumping" character (e. g. exceptional increase of limonene and decrease of *cis*-β-ocimene in July 7), a clear tendency in the accumulation of these compounds including β-phellandrene during flowering period can be observed (Table 3.3, Figure 3.2).

Two oxygenated monoterpene compounds, α-terpineol and geranyl acetate, were also present in appreciable amounts both in leaves (0.69-1.50% and 0.39-0.83% respectively) and stems (0.86-1.48% and 1.01-1.52% respectively). These compounds were minor constituents or not detected at all in the seeds or flowers. On the contrary to monoterpene hydrocarbons, the content of oxygenated compounds, in general, was slightly higher at the initial phases of growth as compared with to the flowering period.

A. Monoterpenes

B. Oxygenated compounds

C. Phthalides

☐ leaves   ■ stems   ▦ flowers   ■ seeds
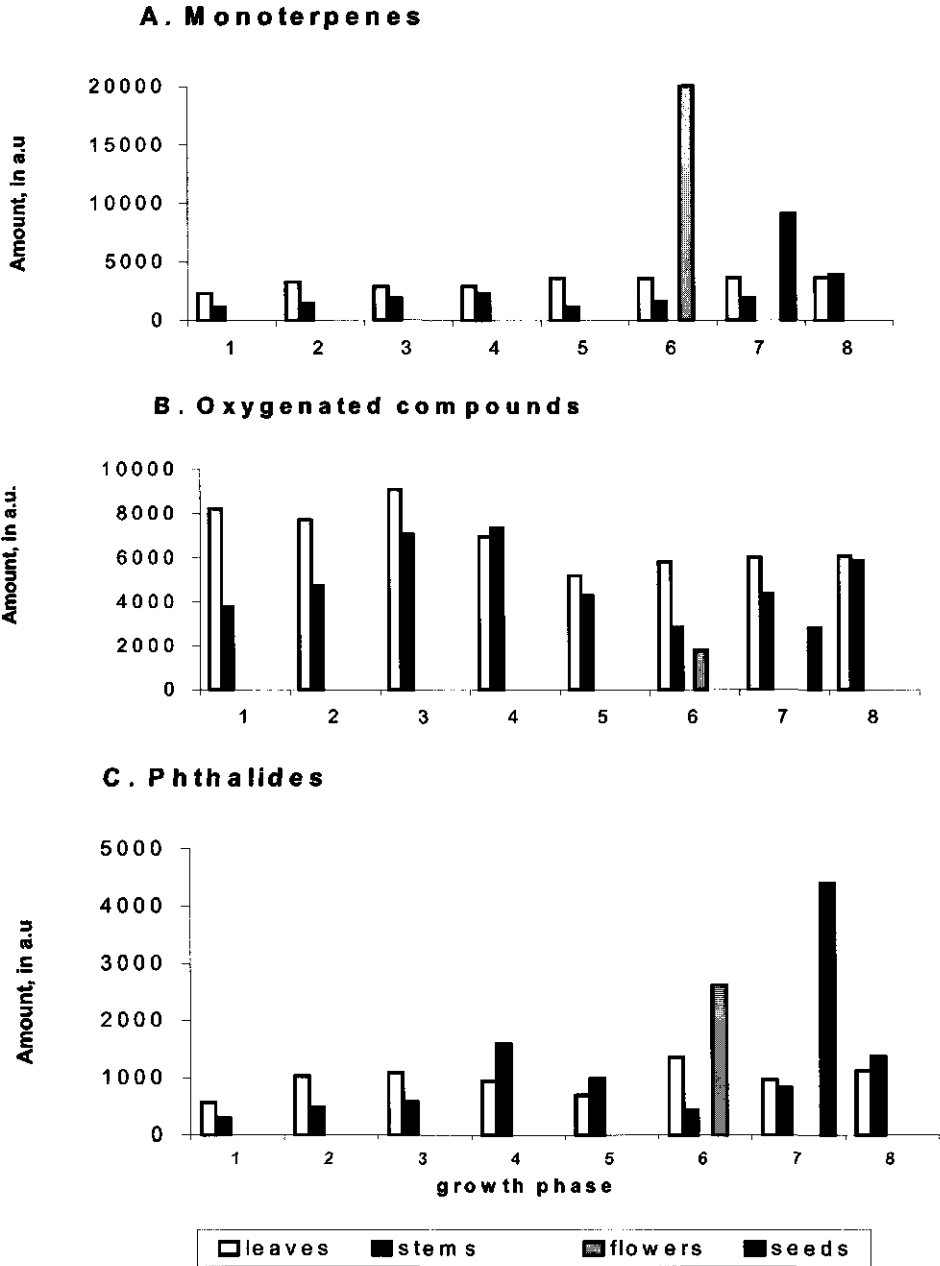
Fig 3.2. Amounts of monoterpenes (A), oxygenated compounds (B) and phtalides (C) in different botanical parts of lovage at different growth phases

a.u.=essential oil yield (%) x percentage content of individual component x 1000 / 100

Minor constituents of lovage

Other constituents identified in different botanical parts of lovage were in minor (<1%) and trace concentrations. A few compounds were detected only in certain parts, e. g., β-farnesene and methyleugenol were detected only in the oils from seeds and flowers, germacrene D was not found in the stems, whereas geraniol was present only in leaves and stems. Three other phthalides were identified in all botanical parts at a concentration of up to 0.50%. A very specific compound of lovage essential oil, namely pentyl cyclohexadiene presents in appreciable amounts in the roots (Venskutonis, 1995) was found at all harvesting periods in all botanical parts, however its concentration did not exceed 1% in the total oil.

CONCLUSIONS

The seasonal changes in the yield of essential oil in lovage leaves was less significant than in the stems, where it increased more than 2 fold from the initial phase of vegetation untill the period of bud formation and subsequent decrease again. The highest yield of oil was found in the seeds followed by the flowers.

The major constituents of essential oils from lovage leaves, stems, flowers and seeds are α-terpinyl acetate, β-phellandrene and Z-ligustilide, constituting from 73 to 88% in the total oil. α-Terpinyl acetate was the dominating compound in leaves and stems (up to 70%), β-phellandrene in seeds and flowers (61.50% and 40.80% respectively).

Seasonal changes in the concentration of individual compounds in lovage leaves and stems were found to be rather complex: in general, the content of monoterpene hydrocarbons and Z-ligustilide was higher during the flowering period, whereas the percentage concentration of oxygenated monoterpene esters and alcohols at this phase was lower as compared to the initial stages of growing.

REFERENCES

Adams, R. P. Identification of Essential Oil Components by GC/MS. Allured Publ. Carol Stream, Ill., USA, 1995.

Bauer, K.; Garbe, D.; Surburg, H. Common Fragrance and Flavour Materials. Preparation, Properties and Uses. VCH Verlagsgesellschaft GmbH Wei*nheim, 1990.*

Blank, I.; Schieberle, P. Analysis of the seasoning-like flavour substances of a commercial lovage extract (*Levisticum officinale* Koch.). Flavour *Fragrance J.* **1993**, 8, 191-195.

Bylaitė, E.; Legger, A.; Roozen, J. P.; Venskutonis, P. R. Dynamic headspace gas chromatography of different botanical parts of lovage (*Levisticum officinale* Koch.). In *Developments in Flavour Science;* Taylor, A. J., Mottram, D. S., Eds.; The Royal Society of Chemistry, UK,1996; pp 66-69.

De Pooter, H. L.; Coolsaet, B. A.; Dirinck, P. J.; Schamp, N. M. GLC of the headspace after concentration on Tenax GC and of the essential oils of apples, fresh celery, fresh lovage, honeysuckle and ginger powder. In *Essential Oils and Aromatic Plants;* Baerheim-Svendsen, A., Scheffer, J. J. C., Eds.; Publisher: Nijhoff/Junk Dordrecht, The Netherlands, 1985; pp 67-77.

Gijbels, M. J. M.; Scheffer, J. J. C.; Baerheim-Svendsen, B. Phthalides in the essential oil from roots of *Levisticum officinale. Planta Medica* **1982**, 44, 207-211.

Heath, H. B. Source book of flavors. AVI Publishing Compamy Inc USA, 1981.

Heath, H. B.; Reineccius, G. Flavor Chemistry and Technology. Macmillan Publishers USA, 1986.

Huopalahti, R.; Linko, R. R. Composition and content of aroma compounds in dill, *Anethum graveolens* L., at three different growth stages. *J. Agric. Food Chem.* **1983**, 31, 331-333.

Cu, J-Q.; Pu, F.; Shi, Y.; Perineu, F.; Delmas, M.; Gaset, A. The chemical composition of lovage headspace and essential oil produced by solvent extraction with various solvents. *J. Essent. Oil. Res.* **1990**, 2, 53-59.

MacLeod, G.; Ames, J. M. Volatile components of celery and celeriac. *Phytochemistry* **1989**, 28, 1817-1824.

Maghami, P. *Culture* et *cueillette des plantes medicinales.* Hachette Paris, 1979.

Official Methods of Analysis of the Association of Official Analytical Chemists; Helrich, K., Ed., publ. by AOAC Inc. USA, 1990, Vol.2, Chapter "Spices and Other Condiments" method 986.21, 999.

Segebrecht, S.; Schilcher, H. Ligustilide: guiding component for preparation of Levisticum officinale roots. *Planta Medica,* **1989**, 55, 572-573.

Senatore, F. Influence of harvesting time on yield and composition of the essential oil of a thyme (*Thymus pulegioides* L) growing wild in Campania (southern Italy). *J. Agric. Food Chem.* **1996**, 44, 1327-1332.

Stahl-Biskup, E.; Wichtmann, E.-M. Composition of the essential oils from roots of some Apiaceae in relation to the development of their oil duct systems. *Flavour Fragrance J.* **1991**, 6, 249-255.

Toulemonde, B.; Noleau, I. Volatile constituents of lovage (*Levisticum officinale* Koch.) In Flavour*s and Fragrances: a World Perspective;* Lawrence, B. M., Mookherjee, B. D., Willis, B. J., Eds; Elsevier Science Publishers B. V. Amsterdam, 1988; pp 641-657.

Toulemonde, B.; Paul, F.; Noleau, I. Phthalides from lovage (*Levisticum officinale* Koch.) In *Flavour Science and Technology;* Martens, M., Dalett, A., Russwurm, H. Eds.; John Wiley & Sons, 1987; pp 89-94.

Uhlig., J.; Chang, A.; Jen, J. J. Effect of phthalides on celery flavor. *J. Food Sci.* **1987**, 52 (3) 658-660.

Venskutonis, P. R. Essential oil composition of some herbs cultivated in Lithuania. In *Flavours, Fragrances and Essential Oils;* vol. 2, Baser, K. H. C. Ed.; Publisher: AREP, Istanbul, 1995; pp 108-123.

COMPOSITION OF ESSENTIAL OIL OF COSTMARY [*BALSAMITA MAJOR* (L.) DESF.] AT DIFFERENT GROWTH PHASES

ABSTRACT

The essential oils from leaves and flowers of costmary, *Balsamita major* (L.) Desf. (syn. *Chrysanthemum balsamita* L.) were analysed at various phases of plant growth. The highest content of oil both in leaves and flowers was determined before full blooming, 1.15 and 1. 34 % (w/w) respectively. 78 volatile compounds have been identified in the oils of *Balsamita major*, of which 58 (19 tentatively, 39 positively) have not been reported in this plant previously. Carvone and α-thujone were found to be dominating compounds constituting from 51.8 to 68.0% and 9.0–16.1% in the total oil respectively. Seasonal variations in the oil compositions were not considerable except for the starting phase (May 25), when the content of carvone was lower and the content of α-thujone and sesquiterpenes higher. The content of sesquiterpenes was about two times higher in flowers than in the leaves. Absolute amount of most components was highest at buds formation period.

INTRODUCTION

Costmary, *Balsamita major* (L.) Desf. (syn. *Chrysanthemum balsamita* L.) Asteraceae, is a large perennial plant of Asian origin with yellow flowers grown in Europe and Asia from the Middle Ages. Fresh and dried leaves of costmary possess strong mint-like aroma and astringent taste. On a domestic scale they have been used to flavour different dishes including fatty minced meat, game, poultry, lamb, fish, curd, potato, soups, sauces, beverages, and cakes. Essential oil of costmary together with some other volatile products was successfully used in creation of flavouring compositions for confectionery (Dapkevičius et al., 1992). However, to our knowledge, commercial cultivation, processing and application of costmary are not widely developed. The publications on essential oils composition of this plant are rather scanty.

Göckeritz (1968) defined two chemotypes of *B. major* according to the dominant terpene in the essential oil: camphor-type, *Balsamita major* (L.) Desf. subsp. *majus* (Desf.) Asch. and carvone-type, *Balsamita major* (L.) Desf. var. *tanacetoïdes* (Boiss.) Fiori. In their literature survey on costmary Bestmann et al. (1984) suggest to distinguish a third chemotype, with camphor and thujone as prevailing components in the essential oil. Göckeritz (1968), Zielinska-Sowicka and Wolbis (1970) have studied the essential oils of camphor chemotype

B. major. They found that the content of oil varied from 0.31% to 1.25% in leaves, and that camphor acts as a major component constituting 72-91% of the total oil content.

Several authors studied the essential oil composition of carvone chemotype *B. major*. Voigt et al. (1938) determined the essential oil content in this costmary chemotype, which varied from 0.38% to 0.64% in dry matter. Jukneviciene et al. (1973) investigated separate anatomical parts of *B. major* at different plant growth phases and found that essential oil content varied from 0.06 to 2.20 ml 100 g^{-1} of dry matter. Three years later Paris et al. (1976) determined 1.3% of essential oil in the flowers of *B. major* and 0.7% in its aerial parts. In both studies carvone showed to be a dominating compound, because it constituted 65-80% of the total oil depending on the growth stage of the plant.

Lukic et al. (1965) and Bestmann et al. (1984) studied the insecticide activity of costmary essential oil. They identified and quantified more than 20 constituents in the essential oil of *Chrysanthemum balsamita* and found each of them possessing insecticide properties against aphids.

Other published studies were focused on particular components of *B. major*, e. g, five compounds were identified in *o*-dihydroxyphenolic fraction of *Chrysanthemum Balsamita* L. var. *tanacetoïdes*, which included caffeic, chlorogenic and ferrulic acids (Tămaş et al., 1989). Todourova and Ognyanov (1989) isolated seven C-9 β-hydroxylated or esterified germacranolides from the flowers of *B. major* population cultivated in Bulgaria and found these lactones differ from the presently known eudesmanolides in a *B. major* population cultivated in Poland.

A study on the seasonal dependence of oil content and composition of main constituents of *Chrysanthemum balsamita* L. was carried out by Strobel et al. (1987), who identified 29 compounds with carvone (55-60%) and thujone (10%) as the major ones. This study showed that the composition of the oil from *Chrysanthemum balsamita* L leaves changes more distinctly during the season than the oil from flower head.

Agrotechnological studies of *B. major* in Lithuanian Institute of Horticulture (Baranauskienė, 1995) showed that the crop of fresh costmary leaves varies from 30 to 45 t ha^{-1}, of which the

yield of dry herb was 16.8%. Therefore, the plant seems to be of interest as a source of natural flavourings. On the other hand, published data on *B. major* essential oil composition and its seasonal changes are rather scarce. Considering these points, the present work was undertaken to study seasonal changes of essential oil composition of *B. major* grown in Lithuania.

MATERIALS AND METHODS

Plant material and isolation of volatile compounds

Plant material was grown in the experimental garden of Lithuanian Institute of Horticulture, Babtai in 1995. The whole herb was harvested every second week, totally 6 times from the end of May till the middle of August as follows: blossoms at the beginning and during full flowering, and leaves at different times of growing phase of the plant. The plants were sorted by botanical part (leaves, stems, flowers), depending on the growth period and naturally dried at room temperature. The oils were obtained by hydrodistillation of the different raw materials in a Clevenger type European Pharmacopoeia apparatus during 2 hrs (Richard et al., 1992). Three replicate analyses were carried out for every oil sample. Simultaneously the content of moisture in air-dried herb was determined by distilling 5 g of the sample with toluene for 1.5 hrs (AOAC, 1990). The yields of oil were expressed in % (w/w) of dry wt.

Identification and quantification of volatile compounds

The essential oils were diluted with Et_2O (1% v/v) and analysed on a Fisons 8160 series gas chromatograph with the FID heated at 250 °C using a DB-5 (J&W, Folsom, CA) (5% phenyldimethyl polysiloxane) fused silica capillary column (30 m length, 0.32 mm i. d., 0.25 μm film thickness). The oven temperature was programmed from 50 °C (5 min hold) to 250 °C at a rate of 3 °C/min with a final hold of 10 min. Helium was used as carrier gas with a linear velocity 35 cm/s. Injector temperature was set up at 260 °C. Four replicate GC analyses were carried out for each essential oil.

Gas chromatography-mass spectrometry (GC/MS) analyses were performed on a combined GC-MS instrument (Varian 3400, Finnigan MAT 95) using BP-5 (Ringwood, Australia) (5%

phenylmethyl polysiloxane) capillary column (25 m length, 0.25 mm i. d., 0.25μm film thickness). The GC oven temperature was programmed from 60 °C to 260 °C at a rate of 3 °C/min. The mass spectrometer was operated in the 70 eV EI mode and scanning from 24 to 300 amu at 0.5 s/dec.

Identification was based mainly on the comparison of retention indices (RI) (Adams, 1995; Davies, 1990) and mass spectra (NIST and the Wageningen Collection of Mass Spectra of natural Products). When available, pure chemicals were used as a reference compounds. The content of the individual constituents was expressed as a peak area percent computed by GC system integrator, and also in arbitrary units, which were calculated by multiplying the percentage content of essential oil by 10^4 (to convert it into mg kg^{-1} of dried herb). The obtained figure is multiplied by the percentage content of a particular constituent and by dividing by 100 (Bylaité et al., 1998).

RESULTS AND DISCUSSION

The yields of essential oil at different harvesting period in costmary leaves and blossoms are given in Table 4.1. The dried leaves and flowers of *B. major* yielded yellowish oil, the content of which varied from 0.18 to 1.34% in the period of monitoring.

Table 4.1 Yields % (w/w of dry weight) of essential oils from costmary at different harvesting periods.

	growth phase and date of harvesting	leaves	blossoms
1.	growing, May 25	0.18±0.03	
2.	growing, June 10	0.71±0.04	
3.	growing, June 25	0.81±0.03	
4.	growing, July 10	0.65±0.03	
5.	buds formation, July 25	1.15±0.04	1.34±0.04
6.	blooming, August 8	0.86±0.03	1.15±0.03

Very low content of the oil (0.18±0.03%) was found in the leaves at the starting phase of growth (May 25). Later essential oil was intensively biosynthesised and the highest amount of it was accumulated during bud formation (July 25) both in leaves (1.15±0.04%) and blossoms

(1.34±0.04%). The results obtained are in agreement with those reported by Strobel et al. (1987), who determined in the fresh leaves of costmary 0.02% and 0.2% in May and second half of July respectively. The content of the oil in the flowers was slightly higher than in the leaves. For comparison it should be said that the content of the oil in blossoms determined earlier in *B. major* from Lithuania by Jukneviciene et al. (1973) was approximately two times higher than in leaves. Very low yields of volatiles were recovered from the stems of the plant (ca. 0.005%), and therefore their composition was not analysed by GC.
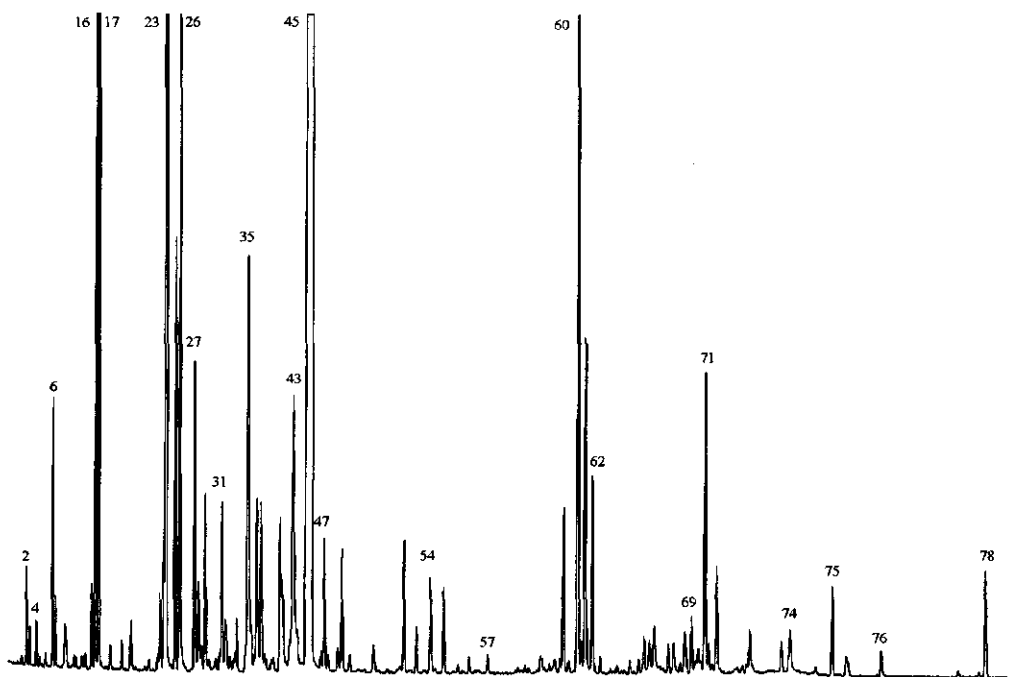
Fig. 4. 1. Gas chromatogram of the essential oil of costmary leaves. See materials and methods for the GC conditions and Table 4.2 for the peak identification.

All 78 identified oil compounds in *B. major* are listed in Table 4.2 in order of their elution time from the capillary BP-5 column. Among them 58 constituents, 39 identified positively (mass spectrum + retention index) and 19 identified tentatively were not reported in *B. major* in previously published papers. Typical gas chromatogram of the essential oil from costmary leaves is presented in Fig. 4.1. Percentage composition of essential oil at different growing phases is presented in Table 4.3; the changes of the amount of the main constituents in a.u.

are shown in Fig. 4.2. Carvone was the dominating constituent at all vegetation periods both in leaves (51.83-68.01%) and flowers (59.5 and 61.67%).
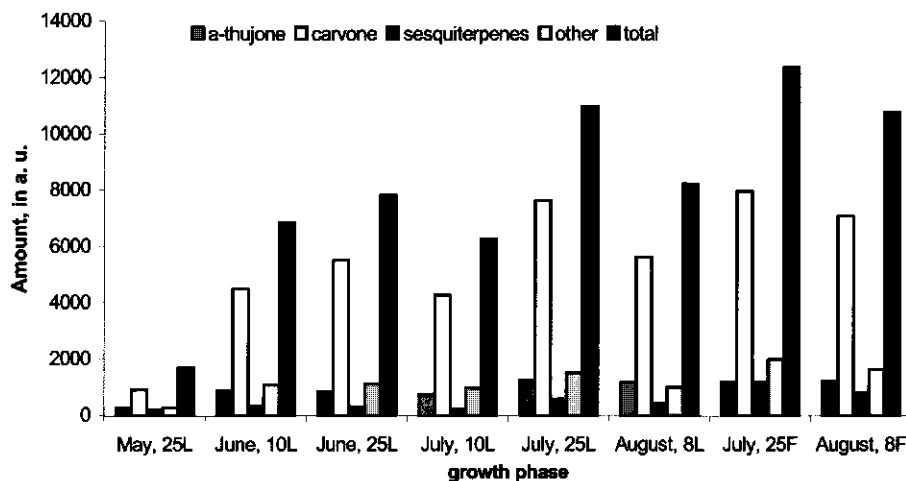
Fig. 4.2 : Amounts of major compounds in leaves (L) and flowers (F) of costmary at different growth phases. a. u. = [essential oil yield (%) × percentage content of individual component × 100]

The differences in the percentage content of carvone at different harvesting time were not considerable except for May 25 when it was the lowest one. The absolute amount (Fig. 4.2) of this constituent was increasing with the increase of the total oil content until buds formation with some exception on July 10, when it was slightly reduced as compared with previous harvesting on June 25. The results presented in Table 4.2 show that it is possible to obtain up to 8 g of natural carvone from 1 kg of dried costmary herb.

The second major compound in the analysed *B. major* was α-thujone, the content of which exceeded 10% in most cases. It is interesting to note that the percentage content of α-thujone, on the contrary to that of carvone, was highest on May 25 (16.1%). However, its absolute amount also was the highest during bud formation, e. g. 1262 a. u. in leaves. According to the two major constituents the chemotype of costmary analysed in our study seems to be similar to the chemotype of *Chrysanthemum balsamita* reported in the work of Strobel et al. (1987).

Table 4.2. Identification data of volatile compounds in costmary leaves and flowers

no.[1]	Constituent	identification	amount[2] (min-max), a. u.	
			leaves	flowers
1.	α-thujene*	a,b	tr	tr
2.	α-pinene	a,b	tr-1.7	17.3-17.4
3.	S-methyl pentanethionate *	a	tr	tr
4.	camphene	a,b	tr-13.8	tr-11.5
5.	benzaldehyde*	a,b	tr	tr
6.	sabinene	a,b	15.7-47.2	64.3-75.9
7.	β-pinene	a,b	tr-16.1	16.1
8/9	myrcene* +2,3-dehydro 1,8-cineole*	a,b	tr-16.3	tr-13.8
10.	isobutyl-2-methylbutanoate*	a	tr	tr
11.	*p*-mentha-1,3,8-triene*	a	tr	-
12.	3-methylbutyl butanoate*	a	-	tr
13.	2-methylbutyl 2-methylpropanoate*	a	tr	tr
14.	α-terpinene*	a,b	tr	tr
15.	*p*-cymene*	a,b	3.6-28.8	21.4-24.2
16/17	limonene+1,8-cineole	a,b	96.7-415.2	28.2-54.2
18.	butyl 2-methylbutanoate*	a	tr	tr
19.	γ-terpinene	a,b	tr	tr
20.	phenyl acetaldehyde*	a,b	tr-12.7	15.0-16.1
21.	3-methylbutyl 2-methylbutanoate*	a	tr	tr
22.	2-methylbutyl 2-methylbutanoate*	a	tr-13.8	60.3-42.6
23.	α-thujone	a,b	289.8-1261.6	1211.4-1239.7
24.	3-methyl 3-butenyl 2-methylbutanoate*	a	-	tr
25.	β-thujone	a,b	15.7-225.4	143.8-187.6
26.	*trans-p*-mentha-2,8-dien-1-ol*	a	39.8-125.4	121.9-138.0
27.	*cis-p*-mentha-2,8-dien-1-ol*	a	8.1-80.5	55.2-67.0
28.	*trans*-pinocarveol	a,b	tr	tr
29.	*cis*-verbenol*	a,b	tr	tr
30.	*trans*-verbenol*	a,b	tr-19.6	tr-18.4
31.	pinocarvone*	a,b	tr-16.1	29.5-32.2
32.	borneol*	a,b	tr	tr
33.	*p*-mentha-1,5-dien-8-ol*	a	tr	tr
34.	terpinen-4-ol	a,b	tr-20.7	10.7-29.9

35.	*trans-p*-mentha-1(7),8-dien-2-ol*	a	tr	tr
36.	α-terpineol*	a,b	tr-17.2	29.5-31.1
37	*p*-cymen-8-ol*	a,b	tr	tr
38.	*cis*-dihydrocarvone*	a,b	5.4-65.6	59.8-72.4
39.	*cis*-piperitol*	a,b	tr	tr
40.	methyl chrysanthemate*	a	-	tr
41/42	*trans*-piperitol* + verbenone*	a,b	8.6-89.7	17.4-21.9
43.	*trans*-carveol*	a,b	tr-26.7	15.0-17.4
44.	*cis-p*-mentha-1(7),8-dien-2-ol*	a	tr-16.3	tr-10.4
45.	*cis*-carveol	a,b	6.3-39.1	26.8-38.0
46.	carvone	a,b	932.9-7637.2	7092.1-7973.0
47	2-phenyl ethylacetate	a,b	tr	tr
48.	*cis*-chrysanthenyl acetate*	a,b	14.-69.0	57.5-113.9
49.	*trans*-carvone oxide*	a,b	9.7-27.6	23.0-24.1
50.	bornyl acetate	a	tr	tr
51	unidentified	c	tr	tr-8.0
52.	*trans*-carvyl acetate	a,b	8.1-25.3	21.9-40.2
53.	α-terpinyl acetate	a,b	-	tr
54.	δ-elemene*	a,b	tr	tr
55.	*cis*-carvyl acetate	a,b	10.7-23.0	44.9-57.6
56.	α-copaene	a,b	tr-16.1	24.1-24.2
57.	*cis*-jasmone*	a,b	tr	tr
58.	β-caryophyllene	a,b	tr	tr
59.	4-*epi*-cubebol*	a,b	16.3-34.5	58.7-120.6
60.	α-muurolene*	a,b	-	tr
61.	β-bisabolene*	a,b	80.1-185.8	116.2-143.4
62.	cubebol*	a,b	tr	tr
63.	*cis*-calamenene*	a,b	27.0-115.0	84.0-113.9
64.	δ-cadinene*	a,b	27.5-51.1	113.9-115.0
65.	*trans*-calamenene*	a,b	tr	tr
66.	cadina-1,4-diene*	a,b	tr	tr
67.	*trans*-nerolidol*	a,b	tr	tr
68.	spathulenol*	a,b	tr	tr
69.	caryophyllene oxide*	a,b	tr	tr
70.	1-*epi*-cubenol*	a,b	6.5-35.7	36.8-38.9

71.	sesquilavandulol (E)*	a,b	-	tr
72.	T-muurolol	a,b	24.1-77.1	248.4-505.2
73.	β-eudesmol*	a,b	-	tr
74.	selin-11-en-4-α-ol*	a,b	9.1-69.0	138.0-142.6
75.	unidentified	c	tr-9.7	21.9-33.7
76.	sesquilavandulyl acetate (E)*	a,b	-	tr
77.	methyl isocostate*	c	tr-19.6	71.3-144.7
78.	6,10,14-trimethyl 2-pentadecanone*	c	-	tr
79.	methyl costate*	c	tr	tr
80.	4-hydroxy 4,5-dihydroisocostate*	c	7.7-28.8	92.0-241.2

[1] – constituents are listed in order of elution from BP5 column

[2] – amounts are calculated from GC-FID peak area percent

a – mass spectrum

b – retention index on DB-5 column

c – not in Adams, not in NIST 98, not in WAU collection

* not previously reported in costmary

tr = trace = <5.0 a. u.

The content of some other constituents in costmary essential oil was higher than 1%: limonene, 1,8-cineole, β-thujone, *trans-p*-mentha-2,8-dien-1-ol, β-bisabolene, T-muurolol and selin-11-en-4-ol (only flowers). Limonene and 1,8-cineole were not separated on DB-5 column, which was used for the analysis of all samples on GC/FID; however, their peaks were baseline separated on BP-5 column by GC/MS analysis. The ratio of limonene and 1,8-cineole calculated from MS data was 1.8 to 1 in the leaves and 1 to 1.4 in the flowers.

The differences in percentage composition between leaves and blossoms were not considerable, except for sesquiterpenes, especially sesquiterpene alcohol T-muurolol, the content of which was considerably higher in blossoms than in leaves at flowering period (July 25, August 8). The absolute amount of sesquiterpenes in costmary flowers was approximately two times higher than in the leaves at the same growing phase. The content of other identified constituents was less than 1% in most cases both in leaves and flowers.

It is interesting to note that the highest percentage content of all identified sesquiterpenes in leaves was much higher at early growth phase (May 25) as compared with later harvesting periods. For instance, the major sesquiterpene β-bisabolene on May 25 constituted 4.45%, whereas on June 10 – 2.33%, June 25 – 1.26%. The absolute amount of sesquiterpenes the

Table 4.3 Percentage composition of essential oils from costmary leaves and flowers at different growth phases (1-6 as in Table 4.1)

component	leaves						flowers	
	1	2	3	4	5	6	5	6
α-pinene	0.25	0.24	0.15	0.22	0.15	0.12	0.13	0.15
camphene	tr	0.14	0.10	0.12	0.12	0.14	tr	0.10
sabinene	0.87	0.65	0.45	0.67	0.41	0.45	0.48	0.66
β-pinene	0.19	0.18	0.13	0.18	0.14	0.13	0.12	0.14
myrcene+2,3-dehydro-1,8-cineole	0.15	0.23	0.10	0.10	tr	tr	tr	0.12
p-cymene	0.20	0.26	0.18	0.25	0.25	0.29	0.16	0.21
limonene+1,8-cineole	5.37	5.42	4.30	4.95	3.61	2.88	2.82	3.75
phenylacetaldehyde	tr	0.13	tr	tr	0.11	tr	0.12	0.13
2-methylbutyl 2-methylbutanoate	tr	0.17	0.12	0.18	0.12	0.13	0.45	0.37
α-thujone	16.10	12.37	10.79	11.83	10.97	13.75	9.04	10.78
β-thujone	2.21	1.28	1.16	1.20	1.09	1.35	1.03	1.06
trans-p-mentha-2,8-dien-1-ol	0.87	2.03	1.82	1.90	1.96	2.05	1.40	1.25
cis-p-mentha-2,8-dien-1-ol	0.45	0.92	0.75	0.80	0.70	0.64	0.50	0.48
trans-verbenol	tr	0.15	tr	0.14	0.17	0.15	tr	0.16
pinocarvone	0.17	0.13	tr	0.18	0.14	0.15	0.22	0.28
cis-dihydrocarvone	0.3	0.69	0.75	0.78	0.57	0.45	0.54	0.52
verbenone+trans-piperitol	0.48	0.49	0.59	0.61	0.78	0.69	0.13	0.19
trans-carveol	tr	0.21	0.33	0.15	0.14	0.18	0.13	0.13
cis-carveol	0.35	0.19	0.36	0.25	0.34	0.38	0.20	0.33
carvone	51.83	63.32	68.01	65.78	66.41	65.36	59.5	61.67
cis-chrysanthenyl acetate	0.79	0.56	0.70	0.59	0.60	0.40	0.85	0.50
carvone oxide	0.54	0.24	0.32	0.20	0.24	0.32	0.18	0.20
trans-carvyl acetate	0.45	0.23	0.24	0.31	0.22	0.11	0.30	0.19
cis-carvyl acetate	0.91	0.15	0.18	0.33	0.20	0.15	0.43	0.39
α-copaene	0.39	0.21	0.12	0.16	0.14	tr	0.18	0.21
4-epi-cubebol	1.10	0.23	0.25	0.28	0.30	0.26	0.90	0.51
β-bisabolene	4.45	2.33	1.26	1.60	1.59	2.16	1.07	1.01
cis-calamenene	1.50	0.82	0.36	0.41	1.00	0.96	0.85	0.73
δ-cadinene	1.53	0.72	0.49	0.55	0.44	0.42	0.85	1.00
1-epi-cubenol	0.36	0.28	0.25	0.11	0.31	0.24	0.29	0.32
t-muurolol	2.19	0.34	0.73	0.47	0.67	0.44	3.77	2.16
selin-11-en-4-ol	0.69	0.35	0.49	0.14	0.60	0.50	1.03	1.24
methyl isocostate	0.43	0.12	0.22	0.19	0.17	tr	1.08	0.62
methyl 4-hydroxy-4,5-dihydroisocostate	0.43	0.15	0.26	0.13	0.25	0.27	1.80	0.80
TOTAL	95.55	95.93	95.96	95.76	94.91	95.52	90.55	92.36

a tr = trace =<0.1%

increased with accumulation of essential oil, however, less considerably as compared with the major compound carvone (Fig. 4.2). Strobel et al. (1987) identified β-cubebene as one of the major sesquiterpenes in costmary, percentage content of which was also decreasing in the period of May-June. We have not identified β-cubebene in our work and its presence in *B. major* seems rather doubtful, because in the chromatogram provided in the paper above mentioned the peak of β-cubebene is shown eluting after β-caryophyllene and β-farnesene peaks. However, retention data obtained from different sources using different polarity capillary columns demonstrate that β-cubebene always elutes earlier than β-caryophyllene and β-farnesene (Davies, 1990; Adams, 1995; Boelens, 1995).

CONCLUSIONS

The highest amount of essential oil in *B. major* leaves and blossoms was accumulated during bud formation. The knowledge about costmary essential oil composition was significantly extended by identifying 58 previously not reported in this plant compounds.

The plants of *B. major* analysed in this study can be defined as belonging to the carvone chemotype, and producing considerable amounts of α-thujone as well.

In general, the compositions of essential oils were quite similar for all harvesting periods except for early growing phase in May when carvone content was lower, while the contents of α-thujone and sesquiterpene higher.

ACKNOWLEDGMENTS

REFERENCES

Adams, R. P. *Identification of Essential Oil Components by GC/MS*; Allured Publishing Corp.: Carol Stream, IL, 1995.

AOAC. *Official Methods of Analysis of the Association of Official Analytical Chemists*; Herlich, K., Ed.; AOAC, Inc.: Arlington VA, 1990; Vol. 2, Chapter "Spices and other Condiments", method 986.21, 999.

Baranauskienė, M. Spicy Plants (Lithuanian); Ūkininko patarėjas: Kaunas, 1995.

Bestmann, H. J.; Claßen, B.; Kobold, U.; Vostrowsky, O.; Klingauf, F.; Strobel, H.; Knobloch, K. Pflanzliche Insektizide II [1]. Das ätherishe Öl aus Blättern des Balsamkrautes, *Chrysanthemum balsamita* L. Insektizide Wirkung und Zusammensetzung. *Z. Naturforsch.* **1984**, 39c, 543-547.

Boelens, M. H. *The complete database of essential oils*; BACIS, The Netherlands, 1991-1995.

Bylaitė, E.; Venskutonis, R. P.; Roozen, J. P. Influence of harvesting time on the composition of volatile components in different anatomical parts of lovage (*Levisticum officinale* Koch.). *J. Agric. Food Chem.* **1998**, 46, 3735-3740.

Dapkevičius, A.; Venskutonis, P. R.; Sūdžienė, S.; Bražaitė R. Use of local aromatic plants in the flavouring of confectionery. In *Chemistry and Technology of Organic Materials and Food* (Lithuanian); Technologija: Kaunas (Lithuania), 1992; pp 54-57.

Davies, N. W. Gas chromatographic retention indices of monoterpenes and sesquiterpenes on methyl silicone and Carbowax 20M phases. *J. Chromatogr.* **1990**, 503, 1-24.

Göckeritz, D. Das ätherishe Öl von *Chrysanthemum balsamita* L. var *balsamita*. *Pharmazie* **1968**, 23, 515-518.

Jukneviciene, G.; Morkunas, A.; Stankeviciene, N. Some biological characteristics and essential oil content of costmary. In *Usefull Plants in Baltic Countries and Belorus, Proc. 2nd Sci. Conf. (Russian)*: Vilnius, 1973; pp 299-303.

Lukic, P.; Savin, K.; Gorunovic, M. Pharmacognostic study of the *Chrysanthemum balsamita*. *Arh. Farm.* **1965**, 15, 249-252.

Paris, M.; Clair, G.; Tillequin, F. Contribution à l'étude chimique de l'huile essentielle de balsamite. *Rivista Ital. Eppos.* **1976**, 58, 473-476.

Richard, H; Noleau, I; Giampoli, P. Techniques d'analyse des épices et aromates. In *Epices et Aromates*; H. Richard (coordonnateur), Tec & Lavoisier: APRIA, 1992; pp 191-211.

Strobel, H.; Knobloch, K.; Ziegler, E. Über die ätherishen Öle von *Chrysanthemum balsamita* L. *Z. Naturforsch.* **1987**, 42c, 502-506.

Tămaş, M.; Sincrăian, A.; Rusu, D. Compuşi o-dihidroxifenolici in *Chrysanthemum balsamita* L. var. *Tanacetoïdes*. *Farmacia (Bucharest)* **1989**, 37, 83-92.

Todourova, M.; Ognyanov, I. V. Sesquiterpene lactones in a population of Balsamita major cultivated in Bulgaria. *Phytochemistry* **1989**, 28, 1115-1117.

Voigt, R. F.; Rogers, C. H.; Fischer, E. B. A pharmacognostic study of *Chrysanthemum balsamita* L. var. *tanacetoïdes* Boiss. together with a study of its volatile oil. *J. Am. Pharm. Ass.* **1938**, 27, 643-654.

Zielinska–Sowicka, R.; Wolbis, M. Étude de la feuille de *Chrysanthemum balsamita* L. *Herba polonica.* **1970**, 16, 286-295.

DYNAMIC HEADSPACE–GAS CHROMATOGRAPHY – OLFACTOMETRY ANALYSIS OF DIFFERENT ANATOMICAL PARTS OF LOVAGE (*LEVISTICUM OFFICINALE* KOCH.) AT EIGHT GROWTH PHASES

This chapter is based on the following articles/manuscripts:

1.Bylaitė, E.; Legger, A.; Roozen, J.P.; Venskutonis, R. "Dynamic headspace gas chromatography of different botanical parts of lovage (*Levisticum officinale* Koch.)". In *"Flavour Science: Recent developments"*, eds. A.J.Taylor, D.S.Mottram, The Royal Society of Chemistry, UK, **1996**, pp 66-69.

2.Bylaitė, E.; Legger, A.; Roozen, J.P.; Venskutonis, R.; Posthumus, M.A. "Dynamic headspace–gas chromatography–olfactometry analysis of different anatomical parts of Lovage (*Levisticum Officinale* Koch.) at eight growth phases." Submitted to the *J. Agric. Food Chem.*

ABSTRACT

Volatiles of five different parts of lovage (leaves, stems, flowers, seeds, and roots) were isolated by dynamic headspace method (DHS) and characterised by GC-FID and GC-olfactometry (GC-O) techniques. Totally, 98 compounds were identified in the samples, 41 of which are reported among lovage volatiles for the first time. Qualitative differences in the composition of DHS constituents in various anatomical parts of the plants were not significant, whereas the amounts of a number of identified volatile compounds were different in leaves, stems, flowers, seeds and roots. Seasonal variations in the composition of headspace volatiles were also determined. Except for roots, β-phellandrene was found to be the most abundant headspace component in all anatomical parts of lovage constituting from 36.50% to 79.28% in the total GC peak area. The sniffing panel characterised effluents from GC column and odour descriptors were attributed to the recognized constituents. α-Pinene and α-phellandrene+myrcene were the most frequently recognized constituents among 11 fractions constituting of 12 identified and 1 unknown compound, which were detected by the members of the sniffing panel. None of the detected constituents was recognized as a lovage key aroma compound.

INTRODUCTION

Studies on the lovage (*Levisticum officinale* Koch.) volatiles obtained by different isolation procedures, such as distillation, solvent extraction, dynamic headspace (DHS) techniques have resulted in the identification of approximately 200 compounds. Most detailed investigations (Toulemonde and Noleu, 1988; Venskutonis, 1995; Bylaite et al., 1998) were performed on the volatiles collected by solvent extraction and distillation. However, the most important goal in aroma analysis is the identification of volatile compounds, which are perceived by the human olfactory system in the gas space above a food (Guth and Grosch, 1993). This can be achieved by using headspace–gas chromatography technique. Static headspace (SHS) provides accurate composition of volatile compounds, which are perceived as a wholesome odour of the product, but it is limited to the level of detection and identification of organic constituents, particularly less volatile substances. DHS techniques

permit to collect bigger amounts of volatile compounds for their qualitative and quantitative characterization by instrumental methods. Nevertheless, many widely used detectors, such as flame ionization (FID), flame photometric (FPD), electron capture (ECD) and mass spectrometric (MS) in numerous cases are not as sensitive for the detection of odourants as the human olfactory system (Acree and Barnard, 1994). For this reason, gas chromatography–olfactometry (GC-O) analysis by using sniffing ports for column effluents seems to be an interesting approach in obtaining important information about the qualitative contribution of individual compounds to the overall flavour of the product. This technique in many cases could be successfully used in determination of so-called key aroma compounds. In agriculture it may also contribute to important decisions concerning selection of cultivars, cultivation and harvesting techniques and storage conditions (Dirinck and De Winne, 1994).

DHS composition of lovage volatiles was reported earlier. Nine components were identified in DHS of fresh lovage (De Pooter et al., 1985) constituting mainly of mono- and sesquiterpenes, while roots composition was dominating by ligustilide and β- phellandrene (Cu et al., 1990). Blank and Schieberle in 1993 reported odourants of the acidic fraction of a commercial lovage extract. Sotolon was the key aroma compound of the acidic fraction due to its characteristic seasoning-like flavour. In our previous report (Bylaite et al., 1996) among 25 volatiles identified in different anatomical parts (leaves, stems, blossoms and seeds) of the plant, some aldehydes and terpenes were considered contributing to lovage flavour.
The present study on DHS-GC and GC-O analysis of volatiles isolated from 5 anatomical parts of plant at different growth stages is aimed at providing a better understanding of the formation of lovage aroma during the vegetation period and to look for the volatile constituents which could have a sensorial contribution to overall odour of lovage.

MATERIALS AND METHODS

Plant material and isolation of volatile compounds

Plant material was harvested in the experimental garden of the Lithuanian Institute of Horticulture from the middle of May until the end of September at 8 different growth phases: 1 = May 15; 2 = May 25; 3 = June 9; 4 = June 16; 5 = June 28; 6 = July 7; 7 = July 19; 8 =

September 21. Raw material was air-dried at 30 °C, packed in glass containers and stored at room temperature in the absence of light.

Essential oils were hydrodistilled in a Clevenger apparatus for 2 h. Isolation of volatile compounds by DHS technique was performed by flushing purified nitrogen gas in order to trap released compounds on a Tenax TA, as described previously (Bylaité et al., 1996).

Identification and quantification of volatile compounds

Volatile compounds were desorbed from Tenax by a thermal desorption (210 °C, 5 min)/cold trap (-120 °C/240 °C) device (Carlo Erba TDAS 5000). The compounds were analyzed on a Carlo Erba MEGA 5300 gas chromatograph equipped with a FID heated at 275 °C by using Supelcowax 10 column (Supelco Inc., Bellefonte, PA), 60 m length, 0.25 mm i.d. and 0.25 µm film thickness. The oven temperature was programmed from 40 °C (4 min hold) to 92 °C at a rate of 2 °C min^{-1} and then to 272 °C at a rate of 6 °C min^{-1} with a final hold of 5 min. The GC effluents were split by the ratio of 1 : 2 : 2 for the FID and for the two sniffing ports respectively, and assessed by a sniffing panel consisting of ten assessors (Linssen et al., 1993). GC–MS analyses were performed on a Varian 3400–Finnigan MAT 95 instrument equipped with a thermal desorption/cold trap device (TCT injector 16200) at 70 ev electron impact ionization mode and scanned from m/z=24 to 300 at 0.5 s decade^{-1}. GC conditions for MS were the same as in GC-FID-O analysis.

Identification was based mainly on the comparison of retention indices (RI) (Adams, 1995; Davies, 1990) and mass spectra (NIST and the Wageningen Collection of Mass Spectra of Natural Products).

RESULTS AND DISCUSSION

Totally, 98 compounds were identified in different anatomical parts of lovage, which are listed in Table 5.1 in order of their elution from Supelcowax 10 column; 41 of them are reported as constituents of lovage aroma for the first time. The amounts of DHS constituents in GC peak area units are presented in Table 5.2.

DHS-GC-FID analysis has revealed considerable differences in the compositions of analyzed anatomical parts of the plant. The dominant quantitatively compound in DHS samples from lovage leaves, stems, flowers and seeds was monoterpene hydrocarbon β-phellandrene. Percentage content of β-phellandrene was 47-66% in the leaves, 36-47% in the stems, 61% in the flowers, 79% in the seeds and 15% in the roots. The content of the second major constituent in the leaves, stems and flowers, α-terpinyl acetate was 9.2-16.1%, 13.7-22.4% and 5.3% respectively. However, this compound was among minor constituents in the seeds and in the roots constituting 0.6% and 2.0% respectively. It is interesting to note that quantitative composition of DHS volatiles from lovage roots was rather different as compared with the other parts. Penthylbenzene was the major constituent (23.6%) followed by β-pinene (20.21%), β-phellandrene (15.11%) and α-pinene (12.75%).

Table 5.1. Volatile compounds identified in DHS-GC of different anatomical parts of lovage

no	compound[a]	leaves	stems	seeds	flowers	roots	earlier reported among lovage volatiles
1	butanal	+	+	+	+	+	+
2	2-methyl prop-2-enal	+			+		-
3	2-methyl butanal	+	+		+	+	+
4	3-methyl butanal	+	+		+	+	+
5	salvene (Z)		+				-
6	pentanal	+	+		+	+	+
7	tricyclene	+	+		+		+
8	α-pinene	+	+	+	+	+	+
9	α-thujene	+	+	+	+		+
10	toluene	+	+	+	+	+	+
11	camphene	+	+	+	+	+	+

12	hexanal	+	+	+	+	+	+
13	β-pinene	+	+	+	+	+	+
14	*iso*-limonene		+				-
15	sabinene	+	+	+	+	+	+
16	undec-1-ene		+			+	+
17	Δ-3-carene			+	+		+
18	myrcene	+	+	+	+	+	+
19	α-phellandrene	+	+	+	+		+
20	α -terpinene	+	+	+	+		+
21	butan-1-ol	+				+	+
22	heptanal					+	+
23	limonene	+	+	+	+	+	+
24	β-phellandrene	+	+	+	+	+	+
25	β-ocimene (Z)	+	+	+	+		+
26	γ-terpinene	+	+	+	+	+	+
27	β-ocimene (E)	+	+	+	+		+
28	pentan-1-ol					+	+
29	*p*-cymene	+	+	+	+	+	+
30	3-methylbuthyl 2-methylbutanoate		+				-
31	terpinolene	+	+	+	+	+	+
32	2-methylbuthyl 2-methylbutanoate	+	+				+
33	octan-2-one		+			+	-
34	octanal	+	+	+	+	+	+
35	3-methylbuthyl 3- methylbutanoate	+	+		+		-
36	2-ethyl hex-2-enal	+			+		-

37	nonan-4-one		+				-
38	pentylcyclohexadiene	+	+	+	+	+	+
39	6-methyl hept-5-en-2-one		+	+	+		-
40	hexan-1-ol			+	+	+	+
41	alloocimene	+	+	+	+		+
42	nonan-2-one					+	-
43	undeca-1,2,5-triene	+					-
44	nonanal		+	+	+	+	+
45	pentylbenzene	+	+	+	+	+	+
46	α-thujone		+				-
47	*p*-mentha-1,3,8-triene			+	+		-
48	*p*-cymenene	+	+	+		+	-
49	β-thujone		+				-
50	acetic acid	+	+	+	+	+	+
51	α-cubebene			+	+	+	+
52	*trans*-sabinene hydrate		+		+		-
53	fenchylacetate	+	+				+
54	α-copaene	+		+	+	+	+
55	decanal	+	+	+	+	+	+
56	hexylbenzene					+	-
57	phellandral	+	+		+	+	+
58	undecan-6-one		+				-
59	propanoic acid	+					-
60	non-2-enal (E)					+	-
61	β-cubebene			+	+		+

62	2-methyl 6-methylene octa-1,7-dien-3-one	+	+	+	+		-
63	linalool acetate	+	+	+	+		+
64	pinocarvone		+				-
65	bornyl acetate	+	+	+	+		+
66	thymol methylether		+				-
67	β-elemene					+	+
68	terpinen-4-ol		+				+
69	β-caryophyllene			+	+		+
70	butanoic acid	+	+		+	+	-
71	α-terpineol				+		+
72	*p*-2-butyl anisole					+	-
73	dec-2-enal (E)					+	+
74	borneol propionate		+				+
75	β-farnesene			+			+
76	acetophenone	+	+	+	+	+	-
77	estragole		+		+		-
78	humulene		+				-
79	α-terpinyl acetate	+	+	+	+	+	+
80	germacrene D			+	+		+
81	pentanoic acid	+	+				-
82	2,6-dimethyl octa-1,5,7-trien-3-ol	+			+		-
83	cuminaldehyde	+	+	+	+		+
84	β-selinene					+	+
85	decan-1-ol	+					-

86	α-phellandrene epoxide			+			-
87	p-mentha-1,3-dien-7-al			+			-
88	δ-cadinene					+	+
89	7-*epi*-α-selinene					+	-
90	methyl salicylate				+		+
91	hexanoic acid	+	+	+	+	+	-
92	dibutyl phtalate	+				+	-
93	octanoic acid	+			+		-
94	nonanoic acid				+		-
95	carvacrol	+					+
96	4-pentylphenol	+		+			-
97	5-hydroxy-p-menth-6-en-2-one	+					-
98	benzoic acid				+		-

[a]Compounds are listed in order of their elution from Supelcowax 10 column

The changes of the total amount of DHS volatile compounds, α-phellandrene and the total amount of hydrodistilled essential oil during vegetation period from May 15 till September 21 is presented in Fig. 5.1. In general, the changes of the amount of the total DHS volatiles and β-phellandrene particularly is in a good agreement with the changes in essential oil content. The concentration of volatiles above seeds containing the highest amount of the essential oil was approximately 3-10 times higher than in the leaves, stems and flowers, while the amount of HS constituents released by lovage roots was very small due to a very low content of essential oil in it. By comparing the composition of essential oil containing α-terpinyl acetate as a major constituent in all anatomical parts of lovage (48-70%) except for flowers and seeds (Bylaitė et al., 1998) with the composition of DHS samples it can be noticed that the amount of β-phellandrene in DHS was several times higher than that of α-terpinyl acetate. Molecular weight of β-phellandrene (FW=136, $bp_{101.3kPa}$=172 °C for α-phellandrene) is lower than that of α-terpinyl acetate (FW=196, $bp_{5.3kPa}$=140 °C) and consequently the volatility of

Table 5.2. Concentration GC peak area units, (n=6) of selected compounds in DHS of lovage at various growth phases (1-8)

compound	leaves								stems								flowers	seeds	roots
	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8	6	7	8
butanal	0.34	0.47	0.58	0.31	0.24	0.44	0.33	0.38	0.60	0.62	0.75	0.63	1.10	0.49	1.53	1.14	1.15	1.20	1.77
3-methyl butanal	0.71	0.96	0.99	0.70	1.06	0.75	0.85	3.96	0.33	0.62	0.33	0.35	0.44	0.52	0.37	1.28	0.21	1.24	0.68
pentanal	0.31	0.31	0.33	0.32	0.35	0.37	0.34	0.33	0.28	0.30	0.34	0.40	0.26	0.28	0.18	0.11	0.45	nd	tr
α-pinene	10.93	12.45	13.25	11.69	4.02	5.46	4.82	7.50	3.52	7.23	9.02	13.03	1.10	1.49	1.53	1.14	4.68	46.80	5.50
α-thujene	1.11	1.39	1.69	1.26	0.84	1.29	1.23	1.30	0.48	0.64	0.87	1.43	0.80	0.76	0.84	0.48	0.78	6.35	nd
toluene	0.65	0.79	0.86	0.81	0.48	0.60	0.57	0.68	0.47	0.55	0.74	0.94	0.80	0.64	0.73	0.50	1.55	2.26	nd
camphene	3.13	3.40	3.24	3.22	1.01	1.32	0.97	1.84	0.70	2.74	2.62	4.10	1.27	2.24	1.87	1.41	1.40	9.12	1.20
β-pinene	2.74	2.92	2.75	2.58	1.26	1.52	1.38	2.26	3.04	4.16	3.60	2.43	2.21	2.05	2.19	2.44	1.81	9.61	8.72
sabinene	16.11	17.19	18	15.78	8.14	11.46	11.90	11.76	4.78	7.00	6.68	7.93	2.23	4.76	4.98	3.64	4.70	15.77	0.12
α-phellandrene	12.10	14.74	15.31	15.43	6.33	10.10	10.65	6.73	1.31	1.55	1.88	2.08	2.22	2.8	2.4	2.50	9.45	38.63	nd
myrcene	26.24	32.26	33.1	36.16	33.66	36.58	32.45	31.17	9.85	10.12	12.61	16.25	13.40	12.25	14.44	19.75	36.17	32.46	0.36
α-terpinene	2.91	3.07	3.25	3.12	1.71	2.45	2.03	1.99	0.37	1.24	1.37	1.55	0.71	1.35	1.15	0.96	1.70	21.24	nd
limonene	5.11	4.18	7.82	9.83	9.81	12.09	13.31	10.56	20.45	30.16	37.02	25.10	26.17	27.44	24.3	26.11	18.94	22.94	1.12
β-phellandrene	319.35	415.55	394.27	333.69	188.25	205.96	205.45	229.27	94.40	106	108	167	88.78	95.79	85.83	78.89	272.71	1775.24	6.52
β-ocimene (Z)	3.53	4.02	5.19	5.95	7.91	8.19	7.71	8.68	0.81	0.93	2.23	1.82	1.46	1.31	1.55	1.31	6.77	32.90	nd
γ-terpinene	2.67	5.64	7.22	7.69	6.18	7.86	12.59	10.37	0.65	0.78	0.93	0.91	1.00	0.99	0.96	0.75	7.04	15.15	tr
β-ocimene (E)	0.51	0.60	1.11	0.54	0.69	0.58	0.63	0.81	0.49	0.36	0.40	0.36	0.49	0.39	0.34	0.30	1.24	12.05	nd
p-cymene	10.72	12.63	14.92	12.79	10.89	18.71	18.1	18.24	5.30	5.41	5.33	6.34	4.62	5.89	5.36	5.52	16.55	51.34	0.49
terpinolene	2.47	3.51	2.18	5.95	3.57	2.68	3.19	3.40	2.99	3.18	4.32	2.66	2.59	2.58	2.52	2.60	0.95	3.65	tr
pentylcyclohexadiene	3.14	4.52	4.75	6.24	6.37	4.67	5.71	2.49	1.25	1.40	1.22	2.06	1.53	1.67	0.68	1.00	2.06	10.15	1.94
alloocimene	2.64	2.75	3.4	4.45	5.08	4.82	5.34	5.17	1.31	1.40	1.82	1.23	1.55	0.69	0.84	0.81	3.96	9.51	nd
undeca-1,3,5-triene	0.45	0.43	0.83	0.41	0.63	0.59	0.59	0.58	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
pentylbenzene	2.11	3.10	2.45	3.52	2.45	2.16	1.59	1.26	1.44	1.72	1.53	2.04	1.75	2.36	1.17	1.29	1.30	7.06	10.20
p-cymenene	1.09	0.84	0.87	1.03	0.54	0.65	0.54	0.72	1.11	1.12	1.23	1.25	1.15	1.16	0.94	0.83	nd	2.47	tr
acetic acid	1.61	2.33	3.35	3.13	2.74	1.84	2.53	2.32	2.35	2.07	2.65	1.65	1.52	1.74	1.79	1.73	0.42	1.84	tr
linalool acetate	0.42	0.32	0.33	0.65	0.27	0.48	0.34	0.45	0.33	0.36	0.42	0.47	0.33	0.30	0.56	0.41	0.74	3.30	nd
bornyl acetate	1.08	1.46	1.10	1.30	0.80	1.23	1.11	0.80	2.21	2.51	2.06	2.35	1.64	2.02	1.87	2.08	0.70	2.98	nd
acetophenone	0.50	0.51	0.82	0.74	0.41	0.51	0.46	0.36	0.59	0.58	0.72	1.07	0.48	0.61	0.64	0.59	0.61	0.64	0.42
α-terpinyl acetate	47.15	58.07	74.83	70.91	63.97	65.33	61.07	63.86	40.51	59.68	61.52	48.13	42.6	34.89	42.19	48.45	23.7	12.41	0.86
2,6-dimethyl octa-1,5,7-trien-3-ol	0.37	0.36	0.39	0.40	0.41	0.43	0.32	0.57	0.57	0.58	0.65	0.46	0.47	0.52	0.56	0.63	0.53	nd	nd
TOTAL	507.84	630.96	648.35	606.05	397.92	432.75	425.1	449.12	209.75	273.72	288.5	351.68	208.75	224	217.55	216.09	446.91	2239.08	43.14
average coefficient of variance (%)	26	25	23	34	35	35	27	33	23	26	23	22	28	30	26	25	29	30	34
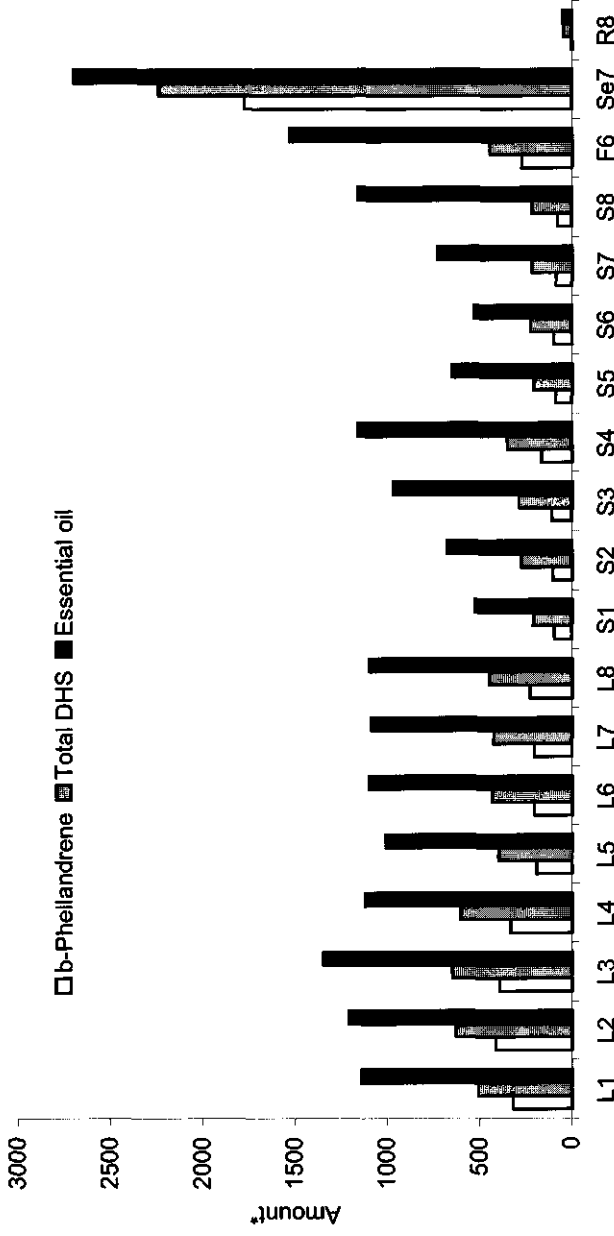
Fig. 5.1 Amount of total DHS volatile compounds, β-phellandrene and total essential oil in lovage leaves (L), stems (S), flowers (F), seeds (Se) and roots (R) at different growth stages. *The amount of total DHS and β-phellandrene is in arbitrary units (GC peak area); the amount of essential oil is in mg 100 g $^{-1}$ w/w of dry weight

monoterpene hydrocarbon is considerably higher than the volatility of acetic acid ester of α-terpineol (Bauer, 1990). It should be emphasized that neither phthalides constituting significant part in the lovage essential oil (Bylaitė et al., 1998) nor sotolon, which was identified as the most important odourant in the acidic fraction of lovage (Blank and Shieberle, 1993) were detected in DHS samples of different anatomical parts of the plant in our study. First of all it was assumed that a small peak of the main phthalide, (Z)-ligustilide in the head space with respect to α-terpinyl acetate (due to an expected lower volatility) may be lost in the relatively intense signals of column bleed and background impurity-peaks in the high temperature range of the chromatogram. However, even selected ion traces for characteristic masses (m/e 148, 161 and 190) did not reveal any trace of (Z)-ligustilide. Also, a sample of the essential oil injected on Tenax, analyzed in the same way as the DHS samples, failed to show ligustilide under analytical TDAS-GC-FID-O conditions. This prompted to determine the retention index for phthalides on Supelcowax column, because the retention indexes for phthalides in literature were available only on nonpolar DB5 column (Adams, 1995).

Table 5.3. Retention indices for the main lovage phthalides

compound	DB-5 (Adams, 1995)	Supelcowax 10
(Z)-3-butylidene phthalide	1668	2558
(E)-3-butylidene phthalide	1711	2672
(Z)-ligustilide	1730	2621
(E)-ligustilide	1790	not detected

The results obtained (Table 5.3) showed that the RI for phthalides on polar column are extremely high and strong temperature program dependent as compared with the RI obtained on nonpolar column. It is obvious from these results that phthalides will not elute from Supelcowax under applied TDAS-GC-FID-O conditions. However, even within the analytical range, elution of phthalides would be unfavorable for sniffing purposes due to the high background odours from the instrument at the elevated temperatures. Apart from these effects, the question remained if phthalides are present in the DHS samples, and in what concentrations. For this purpose one selected DHS sample was run under the same conditions, which were used for the determination of RI's for phthalides in essential oil. However, even in the selected ion trace mode the peak of ligustilide was not visible.

Eleven fractions were detected among the effluents during GC-O analysis of lovage DHS (Table 5.4). Retention times in GC column of such compounds as β-pinene/sabinene and α-phellandrene/myrcene were very close and the panelists perceived these compounds as a unique odour fraction. In general, it could be expected that with the increase of a compound concentration (i. e. its peak area on the chromatogram) the possibility of its detection by sniffing panel also should increase. The results obtained in this study sometimes was in agreement with such statement, however, in many cases this presumption was not accurately followed (Table 5.4). For instance, β-pinene/sabinene were detected by 3 and 4 panelists in the leaves 1 and 2 when their concentration was 18.85 a. u. and 20.75 a. u. respectively. This fraction was not detected in the leaves 5 (9.40 a. u.), and 7 (13.28 a. u.), in the stems 1 (7.84 a. u.), 4 (10.36 a. u.) and 8 (7.44 a. u.), and in the flowers (6.51 a u.). Again, this peak was detected by the 4 panelists in the DHS samples from the seeds where its concentration was the highest (25.38 a. u.). Four members of sniffing panel also detected this fraction in the roots, where the sum of β-pinene and sabinene was only 8.84 a. u. However, in the latter case β-pinene was dominating (8.72 a. u.) in comparison with sabinene (0.12 a. u.), while in all other lovage anatomical parts the amount of sabinene (4.70–18.00 a. u.) was higher than that of β-pinene (1.26–9.61 a. u.). Unfortunately, very little data exists on the odour threshold values of terpenes determined in different media and at various conditions (e. g. temperature), which could be helpful in explanation of such findings. In one of the sources (Table 5.5) odour threshold values for β-pinene and sabinene in water were determined as 140 ppb and 75 ppb, respectively (Fazzalari, 1974). However, this data is not convenient for its interpolation to the GC-O conditions used in our study. In this case the determination of flavour dilution (FD) factors by GC-O (AEDA) would be the most useful techniques in assessing the potential for every particular constituent as it was demonstrated by Blank and Grosch in case of dill (1991) and Guth and Grosch (1993) in case of black tea. Somewhat similar fluctuations can be observed with other in Table 5.5 tabulated constituents, although odour threshold values for the listed monoterpenes are of the same order.

It was already said that phthalides were not detected in DHS by GC-FID due to a very high retention time of these compounds on Supelcowax column and also due to the very low concentrations in DHS samples as it was proved by the aforementioned additional GC/MS analysis. Therefore, sensory evaluation of separately eluting phthalides and consequently their effect on the lovage aroma remains open question. The existing presumption that

Table 5.4. DHS constituents of lovage harvested at different growth phases (1-8), which were detected by sniffing panel; number of panelists simultaneously recognized GC effluent (bold); amount of constituent in DHS, in GC peak area units (in brackets)

RI	constituent	leaves				stems			flowers	seeds	roots
		1	3	5	7	1	4	8	6	7	8
827	unknown	4 (bdt)	5 (bdt)	3 (bdt)	4 (bdt)	0 (bdt)	4 (bdt)	3 (bdt)	6 (bdt)	3 (bdt)	0 (bdt)
878	butanal	3 (0.34)	3 (0.58)	0 (0.24)	4 (0.33)	4 (0.60)	3 (0.63)	3 (1.14)	6 (1.15)	5 (1.20)	0 (1.77)
880	2-methyl prop-2-enal	0 (bdt)	3 (bdt)	0 (bdt)	4 (bdt)	0 (bdt)	0 (bdt)	0 (bdt)	6 (bdt)	0 (bdt)	0 (bdt)
923	2/3-methyl butanal	0 (0.71)	3 (0.99)	3 (1.06)	3 (0.85)	3 (0.33)	5 (0.35)	0 (1.28)	5 (0.21)	0 (1.24)	3 (0.68)
985	pentanal	0 (0.31)	4 (0.33)	3 (0.35)	3 (0.34)	3 (0.28)	3 (0.40)	5 (0.11)	6 (0.45)	0 (bdt)	3 (tr)
1002	α-pinene	3 (10.93)	5 (13.25)	3 (4.02)	4 (4.82)	3 (3.52)	5 (13.03)	5 (1.14)	4 (4.68)	6 (46.80)	3 (5.50)
1102	β-pinene	3 (2.74)	4 (2.75)	0 (1.26)	0 (1.38)	0 (3.04)	0 (2.43)	0 (2.44)	0 (1.81)	4 (9.61)	4 (8.72)
1117	sabinene	(16.11)	(18.00)	(8.14)	(11.90)	(4.78)	(7.93)	(4.98)	(4.70)	(15.77)	(0.12)
1166	α-phellandrene	7 (12.10)	8 (15.31)	4 (6.33)	6 (10.65)	5 (1.31)	5 (2.08)	4 (2.50)	7 (9.45)	6 (38.63)	0 (bdt)
1172	myrcene	(26.24)	(33.10)	(33.66)	(36.58)	(9.85)	(16.25)	(19.75)	(36.17)	(32.46)	(0.36)
1228	β-phellandrene	0 (319.3)	3 (394.3)	0 (188.3)	4 (205.5)	0 (94.40)	4 (167.0)	0 (78.89)	4 (272.7)	5 (1775)	0 (6.52)
1247	(Z)-β-ocimene	0 (3.53)	4 (5.19)	0 (7.91)	3 (7.71)	0 (0.81)	0 (1.82)	0 (1.31)	3 (6.77)	4 (32.9)	0 (0.86)
1690	α-terpinyl acetate	0 (47.15)	3 (74.83)	0 (63.97)	0 (61.07)	0 (40.51)	0 (48.13)	0 (48.45)	3 (23.7)	0 (12.4)	0 (0.86)

bdt = below GC-FID detection threshold; tr = traces

Table 5.5. Odour threshold values and odor descriptors of the detected lovage DHS compounds by GC-O

constituent	odor threshold values	descriptors attributed by panelists	reference descriptors
butanal	0.01-0.03 mg m^{-3} (van Gemert and Nettenbrejjer, 1977); 0.0022 mg dm^{-3}, 0.046 ppm, 0.0092 ppm, 9 ppb: air; 9 ppb, 0.07 ppm: water (Fazzalari, 1974)	chocolate, chemical	fruity, meaty, ethereal (Aldrich, 1993)
2-methyl prop-2-enal	not found	grassy, spicy	
2-methylbutanal	0.004 mg kg^{-1}: water (Guth and Grosch, 1994)	chocolate, spicy, chemical	malty (Masanetz and Grosch, 1998)
3-methylbutanal	0.0019 mg kg^{-1}: water (Rychlik and Grosch, 1996)		malty (Masanetz and Grosch, 1998)
pentanal	0.07 mg m^{-3} (van Gemert and Nettenbrejjer, 1977); 12 ppb (Fazzalari, 1974)	caramel, butter, sour	woody, vanilla, fruity, nutty on dilution (Aldrich, 1993)
α-pinene	0.02 mg m^{-3} (van Gemert and Nettenbrejjer, 1977); 6 ppb: water; 6 ppb, 140 ppb: air (Fazzalari, 1974)	pine, grassy, floral	sharp, pine (Aldrich, 1993)
β-pinene	140 ppb: water (Fazzalari, 1974)		woody, pine (Aldrich, 1993)
		pine, chemical, spicy	
sabinene	75 ppb (Fazzalari, 1974)		woody, terpy, citrus, pine-like with a spice nuance (natural) (Mosciano et al, 1993)
α-phellandrene	13 ppb: water, air (Fazzalari, 1974)		minty, herbaceous, (Aldrich, 1993); citrus, terpenic, slightly green, black-pepper-like (from Givaudan) (Mosciano et al., 1991)
		pine, grassy, chemical	
myrcene	0.01 mg kg^{-1}: water (Masanetz and Grosch, 1998)		sweet, balsamic, plastic (Aldrich, 1993); metallic, herbaceous (Masanetz and Grosch, 1998)
β-phellandrene	0.036 mg kg^{-1}: water (Masanetz and Grosch, 1998)	grassy, chemical	Terpene-like (Masanetz and Grosch, 1998)
(Z)-β-ocimene	not found	mushrooms, musty, chemical	tropical, green, terpy and woody with vegetable nuances (natural) (Mosciano et al, 1990)
α-terpinyl acetate	not found	floral sweet	herbal, citrus, spicy, woody, floral, waxy and clean (natural) (Mosciano, 1997)

particularly ligustilide are the important aroma constituents of lovage roots (α-terpinyl acetate is considered as one of the most important aroma constituents in the leaves) (Gijbels et al., 1982; Toulemonde et al., 1987; Segebrecht and Schilcher, 1989; Bauer, 1990) should be tested by using nonpolar column. However, it should be noted that the success of such tests is also doubtful because phthalides were not detected in DHS on Supelcowax column even by using favorable for their elution and high sensitivity selected ion GC/MS conditions.

Several speculations could be provided to explain the results obtained. One of the reasons could be possible limitations in the recognition abilities of the members of the sniffing panel. As it was mentioned, the panelists were trained persons, however their experience could be not sufficient in this kind of experiments. Second, and most likely more important, sniffing and accurate assessment of lovage DHS effluents is rather difficult task because of a large number of compounds eluting from the column at short time intervals. It seems that the applicability of the GC-O method in such cases becomes less effective. It is also important to note that during smelling of a wholesome lovage aroma, olfactory organs are affected by a great number of compounds at the same time and in this case a synergistic and/or sensor-stimulating effect between different compounds could occur. For instance, it is reasonable to suppose that the sum of all phthalides in the HS could have detectable impact on aroma, whereas the amount of a separately eluting compound could be insufficient for the detection and/or recognition. It is well known that the mechanisms of odour transmission, sensing and recognition are extremely complex and still vaguely understood processes.

Odour active constituents were characterized by using odour descriptors, which are provided in Table 5.5 together with the available odour threshold values. The specific flavour of lovage roots is described in different sources by very general and abstract characteristics, such as medium aromatic (Heath and Reineccius, 1986) or by attributing several descriptors, e. g., strong impact warmly aromatic with sweet, yeasty, musky, lemon-like, celery-like notes (Heath,1981).

Totally, the list of 17 descriptors was prepared for the effluents of lovage DHS during the four preliminary panel sessions. Data provided in Table 5.5 also demonstrates that there is a significant dispersion in describing the odour of the same compound. Sometimes odour

descriptors provided in various sources are very controversial. For instance, the odour of butanal was characterized with such strikingly different descriptors as fruity, meaty and ethereal (Aldrich, 1993), that of α-phellandrene – minty, herbaceous, (Aldrich, 1993), citrus, terpenic, slightly green, black-pepper-like (Mosciano et al., 1991).

An interesting observation could be made concerning (Z)-β-ocimene, which among others was attributed such descriptor as "mushroom-like". Mushroom-like odour has also been attributed to the different anatomical parts of lovage (Baranauskienė, 1995). Odour threshold values also can vary in a wide range depending on testing media, conditions and other factors. All these aspects make accurate assessment of such complex flavours as lovage rather difficult.

CONCLUSIONS

GC/MS analysis of a great number of DHS samples from different anatomical parts of lovage has lead to the identification of 41 constituent previously not reported in this plant. The dominant constituent was β-phellandrene in the most of DHS samples, however, its impact on the lovage aroma does not seem to be the most significant. In general, DHS-GC-O analysis of all anatomical parts of lovage did not reveal specific key aroma compounds, which could be unambiguously used for the characterization of lovage aroma. It seems, that according to the proposed classification of the foods into the four aroma complexity groups (Belitz and Grosch, 1987), lovage, on the contrary to many other aromatic and spicy plants (e. g. mints, dill, caraway, cinnamon, etc.), which odour can be fully characterized by 1-3 constituents (1st or 2nd group), belongs to the 3rd or even 4th group, when a great number of different compounds is needed to simulate original aroma.

ACKNOWLEDGMENTS

experiments and Dr. M. Baranauskienė and Dr. P. Viškelis from Lithuanian Institute of Horticulture for providing lovage plants.

REFERENCES

Acree, T. E.; Barnard, J. Gas chromatography-olfactometry and Charm Analysis. In *Trends in Flavour Research*; Maarse, H., Van der Heij, D. G., Eds.; Elsevier Science Publishers B. V.: Amsterdam, 1994; pp 211-220.

Adams, R. P. *Identification of Essential Oil Components by GC/MS*. Allured Publising Corp.: Carol Stream, IL., 1995.

Aldrich *Flavours & Fragrances*. International Edition, 1993.

Baranauskienė, M. *Spicy Plants (Lithuanian)* Ūkininko patarėjas: Kaunas, 1995.

Bauer, K.; Garbe, D.; Surburg, H. *Common Fragrance and Flavour Materials. Preparation, Properties and Uses*. VCH Verlagsgesellschaft GmbH: Weinheim, 1990.

Belitz, H.-D; Grosh, W. *Food Chemistry*. Springer-Verlag: Berlin, Heidelberg, 1987.

Blank, I.; Grosch, W. Evaluation of potent odourants in dill seed and dill herb (*Anethum graveolens* L.) by aroma extract dilution analysis. *J. Food Sci.* **1991**, 56, 63-67.

Blank, I.; Schieberle, P. Analysis of the seasoning-like flavour substances of a commercial lovage extract (*Levisticum officinale* Koch.). *Flavour Fragrance J.* **1993**, 8, 191-195.

Bylaitė, E.; Legger, A.; Roozen, J. P.; Venskutonis, P. R. Dynamic headspace gas chromatography of different botanical parts of lovage (*Levisticum officinale* Koch.) In *Developments in Flavour Science;* Taylor, A. J., Mottram, D. S., Eds.; The Royal Society of Chemistry: UK, 1996; pp 66-69.

Bylaitė, E.; Venskutonis, P. R.; Roozen, J. P. Influence of harvesting time on the composition of volatile components in different anatomical parts of lovage (*Levisticum officinale* Koch.). *J. Agric. Food Chem.* **1998**, 46, 3735-3740.

Compilation of Odour and Taste Threshold values Data. Fazzalari, F. A., Ed.; ASTM Data Series DS 48A, American Society for Testing and Materials: Philadelphia, Pa.

Cu, J-Q.; Pu, F.; Shi, Y.; Perineu, F.; Delmas, M.; Gaset, A. The chemical composition of lovage headspace and essential oil produced by solvent extraction with various solvents. *J. Essent. Oil. Res.* **1990**, 2, 53-59.

Davies, N. W. Gas chromatographic retention indices of monoterpenes and sesquiterpenes on methyl silicone and Carbowax 20M phases. *J. Chromatogr.* **1990**, 503, 1-24.

De Pooter, H. L.; Coolsaet, B. A.; Dirinck, P. J.; Schamp, N. M. GLC of the headspace after concentration on Tenax GC and of the essential oils of apples, fresh celery, fresh lovage, honeysuckle and ginger powder. In *Essential Oils and Aromatic Plants;* Baerheim-Svendsen, A., Scheffer, J. J. C., Eds.; Publisher: Nijhoff/Junk Dordrecht, The Netherlands, 1985; pp 67-77.

Dirinck, P.; De Winne, A. Advantages of instrumental procedures for measurement of flavours characters. In *Trends in Flavour Research*; Maarse, H., Van der Heij, D. G., Eds; Elsevier Science Publishers B. V.: Amsterdam, 1994; pp 249-258.

van Gemert, L. J.; Nettenbreijer, A. H. *Compilation of Odour Threshold Values in Air and Water.* CIVO-TNO: Zeist, The Netherlands, 1977.

Gijbels, M. J. M.; Scheffer, J. J. C.; Baerheim-Svendsen, B. Phthalides in the essential oil from roots of *Levisticum officinale. Planta Medica* **1982**, 44, 207-211.

Guth, H.; Grosch W. Identification of potent odourants in static headspace samples of green and black tea powders on the basis of aroma extract dilution analysis (AEDA). *Flavour Fragrance. J.,* **1993**, 8, 173-178.

Guth, H.; Grosch W. Identification of the character impact odourants of stewed beef juice by instrumental analyses and sensory studies. *J. Agric. Food Chem.,* **1994**, 42, 2862-2866.

Heath, H. B. *Source book of flavours.* AVI Publishing Co., Inc.: New York, 1981.

Heath, H. B.; Reineccius, G. *Flavour Chemistry and Technology.* Macmillan Publishers: Riverside, NJ, 1986.

Linssen, J. P. H.; Janssens, J. L. G. M.; Roozen, J. P.; Posthumus, M. A. Combined gas chromatography and sniffing port analysis of volatile compounds of mineral water packed in laminated packages. *Food Chem.,* **1993**, 46, 367-371.

Masanetz, C.; Grosch, W. Hay-like off-flavour of dry parsley. *Z. Lebensm. Unters. Forsch.* A, **1998**, 206, 114-120.

Mosciano, G. Organoleptic characteristics of flavour materials, *Perfum. Flavour.,* **1997**, 22, 75-78.

Mosciano G.; Fasano M.; Cassidy J.; Connelly C.; Mazeiko P.; Montenegro A.; Michalski, J.; Sadural S. Organoleptic characteristics of flavour materials. *Perfum.Flavour.* **1993**, 18, 43-45.

Mosciano, G.; Fasano, M.; Michalski, J.; Sadural S. Organoleptic characteristics of flavour materials. *Perfum. Flavour.* **1990**, 15, 69-73.

Mosciano, G.; Fasano, M.; Michalski, J.; Sadural S. Organoleptic characteristics of flavour materials. *Perfum. Flavour.* **1991**, 16, 49-55.

Rychlik, M; Grosch W. *Lebensm. Wiss. Technol.,* **1996**, 29, 515-525.

Segebrecht, S.; Schilcher, H. Ligustilide: guiding component for preparation of *Levisticum officinale* roots. *Planta Medica* **1989**, 55, 572-573.

Toulemonde, B.; Noleau, I. Volatile constituents of lovage (*Levisticum officinale* Koch.) In *Flavours and Fragrances: a World Perspective;* Lawrence, B. M., Mookherjee, B. D., Willis, B. J., Eds; Elsevier Science Publishers B. V.: Amsterdam, 1988; pp 641-657.

Toulemonde, B.; Paul, F.; Noleau, I. Phthalides from lovage (*Levisticum officinale* Koch.) In *Flavour Science and Technology;* Martens, M., Dalett, A., Russwurm, H. Eds.; John Wiley & Sons: New York, 1987; pp 89-94.

Venskutonis, P. R. Essential oil composition of some herbs cultivated in Lithuania. In *Flavours, Fragrances and Essential* AREP, Istanbul, 1995; pp 108-123

EMULSIFICATION OF CARAWAY ESSENTIAL OIL IN WATER USING LECITHIN AND β-LACTOGLOBULIN: EMULSION STABILITY AND PROPERTIES OF THE RESULTING OIL/AQUEOUS INTERFACE

ABSTRACT

The stability and droplet size of protein- and lipid-stabilised emulsions of caraway essential oil and the amount of protein on the emulsion droplets were both investigated. The amounts of added protein (β-lactoglobulin) and lipid (phosphatidylcholine from soyabean (sb-PC)) were varied and the results were compared with those obtained from emulsions of a purified olive oil. In general, emulsions with triglyceride oil proved to be more stable than those made with caraway essential oil as the dispersed phase. However, the stability of the emulsions can be improved considerably by adding sb-PC. An increase in the protein concentration also promoted emulsion stability. The use of ellipsometry to measure both the adsorption of the lipid from the oil and the protein from the aqueous phase at the oil/water interface was introduced in this study. Independently of the concentration used, a monolayer coverage of sb-PC was almost achieved at the caraway oil/aqueous interface. However, at the olive oil/aqueous interface, the presence of only a small amount of sb-PC led to an exponential increase in layer thickness with time to beyond a monolayer coverage. The amount of β-lactoglobulin adsorbed at the caraway oil/aqueous interface was the same as at the olive oil/aqueous interface and corresponded roughly to a protein monolayer.

INTRODUCTION

Whey proteins are frequently used as functional and nutritional ingredients in food systems (Morr, 1982: De Wit, 1989; Kinsella et al., 1989). One of the novel applications of whey proteins is to use them as a coating or "wall" material in micro-encapsulation. This process involves entrapping droplets or particles of sensitive or bioactive material into thin films of coating material (Rosenberg and Young, 1993; Sheu and Rosenberg, 1993; Faldt and Bergenstahl, 1996a,b; Kim and Morr, 1996; Kim et al., 1996; Moreau and Rosenberg, 1996). Food ingredients such as aroma and flavour compounds, oils and fats, vitamins and essential oils are commonly encapsulated by spray drying (Dziezak, 1988; Jackson and Lee, 1991). The emulsification process, when liquid material (core) is dispersed into a solution of encapsulating (wall) material, is one of the key factors that controls the retention of flavour during the drying process (Rish and Reineccius, 1988). To our knowledge the emulsification of flavouring substances, and essential oils in particular, has not been thoroughly investigated. This work therefore focuses on protein- and lipid-stabilised emulsions of caraway essential oil. The stability and droplet size of the resulting emulsion as well as the amount of protein on the emulsion droplets were investigated. The amounts of added protein (β-lactoglobulin) and lipid (phosphatidylcholine from soyabean (sb-PC)) were varied and the results were compared with those obtained from emulsions of a triglyceride oil, i. e. purified olive oil (~74% triolein). Further information on the properties of the oil/aqueous interface and how it is

affected by the presence of protein and lipid was obtained using ellipsometry. This technique was used to determine the adsorption of the lipid from the oil and the protein from the aqueous phase at the oil/aqueous interface. It was found that some useful information can be obtained from the ellipsometry data. The protein used, β-lactoglobulin, is known to bind a number of hydrophobic compounds (Sawyer et al., 1998) and it has been suggested that it has a biological function as a transport protein (Brownlow et al., 1997). In combination with phosphatidic acid, the protein is capable of masking bitter tastes (Kurihara and Katsuragi, 1993) and therefore it is very suitable for encapsulating essential oils.

The good functional properties of proteins, i. e. their solubility, viscosity, emulsification, as well as their ability to interact with water, small ions and other polymers at the oil/water interface, make them particularly suitable for stabilising the emulsion droplets formed during homogenisation (Walstra, 1988; Kinsella, 1990; Morr and Ha, 1993). The ability of whey protein to adsorb at the oil/water interface and stabilise emulsions has been investigated extensively (Pearce and Kinsella, 1978; Shimizu et al., 1985; Kinsella and Whitehead, 1989). However, during or after emulsification some of the initially adsorbed proteins may be displaced from the interface by other (less abundant) more surface-active components, such as other proteins, lipids or surfactants (Courthaudon et al., 1991a,c; Chen and Dickinson, 1993; Tomas et al., 1994). The competitive adsorption of different surface-active components, and thus the composition of the stabilising layer of the dispersed phase, is affected by the strength of the interaction between surface-active species, the ratio between the total emulsifier present and the area of the fat surface formed. No preferential adsorption of one of the proteins at the newly made oil/water interface was found when a mixture of α-lactalbumin and β-lactoglobulin was used as an emulsifier (Courthaudon et al., 1991c). Even after storage, almost no displacement of one protein by the other occurred (Dalgleish et al., 1991). The presence of oil-soluble surfactants in β-lactoglobulin-stabilised emulsions increased the surface coverage of protein at low surfactant concentration, while the protein surface concentration was reduced at high surfactant concentration (Dickinson et al., 1993). This is in contrast with the results from a similar study, where instead a water-soluble surfactant was added during the preparation of β-lactoglobulin-stabilised emulsions (Courthaudon et al., 1991d). It was found that the protein was completely displaced by the surfactant at an emulsifier-to-protein molar ratio of 8:1. In general, surfactants or lipids that have the optimal balance between hydrophilic and hydrophobic properties to be a good emulsifiers also form a lamellar crystalline phase (Bergenståhl and Claesson, 1990). Lecithin from egg yolk or soyabean, where the main constituent is phosphatidylcholine (PC), is such an emulsifier and is therefore one of the most commonly used ones. It is also one of the few food emulsifiers in common use which is accepted by consumers and legislators as being genuinely natural (Dickinson and Iveson, 1993). The purity of lecithin and the way it is added (e.g. to the oil or

to the water phase) are certain to affect the interaction with proteins, which in turn affects the emulsion stability. This has been studied by Yamamoto and Araki (1997), who compared the interfacial behaviour of β-lactoglobulin, in the presence of lecithin in the water or in the oil phase, with the stability of the corresponding emulsions. In the presence of protein, crude lecithin was found to increase the stability of the emulsion and lower the interfacial tension more effectively than a pure lecithin preparation. When (crude) lecithin was added to the oil phase, the interfacial tension was found to decrease, and the emulsion stability increased in comparison with an emulsion which had the lecithin dispersed in the water phase.

MATERIALS AND METHODS

Materials

The β-lactoglobulin sample (DFIblg1, containing 90.2% β-lactoglobulin, 4.7% moisture, 3.1% ash, 0.4% α-lactalbumin and 0.4% fat) was kindly provided by Borculo Domo Ingredients, The Netherlands, and was used without further purification. The olive oil was a highly purified sample (SS-1018 CPO olive oil, lot no 8070:2 from Larodan Fine Chemicals, Sweden) and was shown to be free of monoglyceride, diglycerides and free fatty acids by HPLC. The fatty acid composition expressed as peak area percentage was 10.6% C16:0, 3.5% C18:0, 73.8% C18:1, 8.1% C18:2 and 0.6% C18:3. Commercial caraway essential oil was purchased from Frey & Lauhenstedtulzburg, Germany. Gas chromatography analysis of caraway essential oil has shown that it is mainly (~98% of total oil) composed of two hydrocarbons: limonene and carvone in a 1:1 ratio. Soyabean phosphatidylcholine (sb-PC) was a high purity grade sample (Batch 1-7-9027, containing 94.2% phosphatidylcholine, 1.2% lysophosphatidylcholine) Epikuron 200 obtained from Lucas Meyer, Germany. Epikuron 200 has previously been found to have the following fatty acid composition: C16:0 = 13.3%, C18:0 = 3.0%, C18:1 = 10.2%, C18:2 = 66.9% and C18:3 = 6.6% (Shinoda et al., 1991). Buffer solutions were prepared from analytical grade reagents in double distilled water passed through a Milli-Q water purification system (Millipore Corporation).

Methods

Preparation of emulsions

The aqueous phase was prepared by dissolving 1.17% or 2.35% β-lactoglobulin in 60 mM phosphate buffer at pH 6.7, the solution then being extensively dialysed against the same buffer for 24 hours. Sodium azide was added to each emulsion (0.01%) as a preservative. Different quantities of phosphatidylcholine (PC) were dissolved in the oil phase (caraway and

olive oils) before homogenisation to give a concentration range of 0.2–0.8% in the final emulsion. This corresponds approximately to a molar ratio (M_r) of lipid to protein ranging from 5 to 22. Oil-in-water emulsions, containing 1 or 2% β-lg, 15% oil and 0–0.8% sb-PC, were prepared at room temperature. The initial emulsification was done at 31 000 rpm for 7 min using a Heidolph Diax 900 Homogenizer (Heidolph, Germany), fitted with a 10G Homogenizer Tool. Homogenisation was completed by sonicating the emulsions for 100 s at 40 W output effect in a Vibra Cell sonicator (Sonics & Materials Inc., CO, USA) with a micro-tip Mod No V1A.

Average size of emulsion droplets

Average droplet size in freshly made and diluted (1:20) emulsions was determined by light microscopy with a Zeiss Axioplan Microscope (Carl Zeiss, Jena, Germany) using an ocular with a magnification of ×40. The images were collected with a video camera (total magnification about ×400) and the particle sizes were later calculated manually from the images.

Protein load on emulsion droplets

In order to determine the amounts of protein adsorbed at the oil/water interfaces, the emulsions were first centrifuged at 3500 rpm (~2800 *g*) for 30 min at 25°C to separate the oil droplets from the aqueous phase. Serum was then withdrawn with a syringe and the protein content was analysed using a modified Lowry method (Lowry et al., 1951). The total amount of adsorbed protein was estimated from the amount of protein in serum after centrifugation and the amount used to make the original emulsions.

Emulsion stability (creaming)

Emulsion stability was evaluated by measuring the extent of gravitational phase separation. This method has been suggested as an adequate predictor of short-term emulsion stability of emulsions made with milk proteins (Fligner et al., 1990). For the measurements, emulsion samples were transferred into test tubes with an inner diameter of 5.75 mm and a length of 22 cm. The tubes were tightly capped and stored at room temperature. When the creaming process was complete, we measured the thickness of the cream layer in the test tubes.

Properties of the oil/water interface

The properties of the oil/water interface were characterised *in situ* by ellipsometry (Azzam and Bashara, 1987). When (elliptically) polarised light is reflected against a surface, the change in the state of polarisation, expressed as amplitude ratio, Ψ, and phase shift, Δ, can be measured with an ellipsometer. For a clean interface between a solid and a liquid, liquid and air or between two liquids, Ψ and Δ will give the refractive index of the material. When a film is present at the interface, the measured Ψ and Δ values are also dependent on the thickness and refractive index of the film. We used ellipsometry here to characterise the interface between two oils (caraway oil and olive oil) and water with and without the presence of phosphatidylcholine in the oil phase and a protein dissolved in the aqueous phase. Our measurements were performed in a simple circular glass cuvette (diameter 55 mm) to which 20 ml of buffer had been added. A Teflon cylinder (wall thickness 2 mm) was fitted into the glass cell until it touched the aqueous surface. The oil (10 ml) was then added on top of the aqueous phase, giving an oil/aqueous interface area of about 20 cm^2. This ensured that the meniscus at the oil/water interface was as flat as possible. The ellipsometer (Optrel Multiskop; Optrel, Berlin, Germany), described in detail by Harke et al. (1997), was arranged in such a way that the incident light was reflected at the air/oil and oil/aqueous interfaces as shown in Fig. 6.1. With this setup it is possible to measure the properties of both the air/oil interface and the oil/aqueous interface. In this study we only considered the oil/water interface and, as the oil film was thick enough, it was easy to separate out the reflected light from this interface. To facilitate the addition of protein to the aqueous phase, a smaller glass beaker was connected to the glass cell via a Teflon stopcock. Before 2 ml of the protein stock solution was added, the same volume of buffer was withdrawn from the glass cell via the connected vessel. Thus the interface was disturbed as little as possible.

Calculation of the optical properties of the interfaces

To justify the analysis of the data and to demonstrate the power of the method, the basic theory of the method is presented. Ellipsometry is based on the measurements of changes in ellipticity of polarised light upon reflection at a surface. Polarised light in general can be described as two superimposed light waves, the p and s components, where each of the two waves resides in a plane perpendicular to the other. For plane-polarised light, the phase shift between these two light waves is either zero or multiples of 180°. In the general case of elliptically polarised light, the phase shift is different. The reflection of light at each interface can be described by the Fresnel reflection coefficients, r_p and r_s for the p and s components, respectively. Similarly, transmission coefficients t_p and t_s can be described. These coefficients depend on the angles of incidence and refraction and the refractive indices of the media on

both sides of the interface. For an interface between two dielectric media, the reflection coefficients are real quantities, while they become complex for a semiconductor, a metal or any material that absorbs light at the wavelength used. For an interface the ratio $\rho = r_p/r_s$ is related to the ellipsometer angles Δ and Ψ as

$$\rho = \frac{r_p}{r_s} = \tan\Psi e^{i\Delta} = \tan\Psi(\cos\Delta + i\sin\Delta) \qquad [6.1]$$

The optical layer model used to evaluate our data is shown in Fig. 6.1. For each of the interfaces a reflection coefficient can be derived. They can be expressed as

$$r_{pij} = \frac{n_j\cos\phi_i - n_i\cos\phi_j}{n_j\cos\phi_i + n_i\cos\phi_j} \qquad [6.2]$$

$$r_{sij} = \frac{n_i\cos\phi_i - n_j\cos\phi_j}{n_i\cos\phi_i + n_j\cos\phi_j} \qquad [6.3]$$

$$t_{pij} = \frac{2n_i\cos\phi_i}{n_j\cos\phi_i + n_i\cos\phi_j} \qquad [6.4]$$

$$t_{sij} = \frac{2n_i\cos\phi_i}{n_i\cos\phi_i + n_j\cos\phi_j} \qquad [6.5]$$

Here the refractive index and angle of reflection (or refraction) for medium (i) are n_i and ϕ_i, respectively. The reflection coefficients between two dielectric media are real numbers and thus from equation [6.1] it follows that Δ can only assume the values 0° (or 360° depending on the definition of the angles) or 180°. For such an interface we will only measure a change in Ψ when the refractive indices of the media or the angle of incidence is changed. The value of Δ will be 180° degrees below and 0° or 360° above the Brewster angle. At the Brewster angle the reflection coefficients are zero. It can be shown that the largest sensitivity of the measured angles Δ and ψ is closest to the Brewster angle (Azzam and Bashara, 1987). We then introduced a dielectric thin film (less than the wavelength of the light) between the two dielectric media. Now the reflection coefficients have to take into account the multiple reflection that occurs within the film (cf. McCrackin et al., 1963). The reflection coefficients can, for this case, be expressed using the notation in Fig. 6.1, as:

$$r_{pf} = \frac{r_{1p} + r_{f2p}e^{iD}}{1 + r_{1p}r_{f2p}e^{iD}} \qquad [6.6]$$

$$r_{sf} = \frac{r_{1f_s} + r_{f2_s}e^{iD}}{1 + r_{1f_s}r_{f2_s}e^{iD}}$$ [6.7]
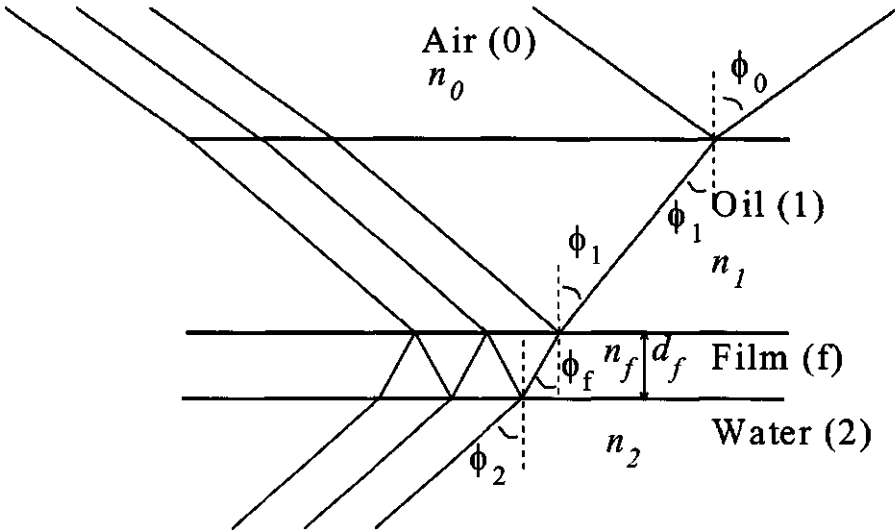
Fig. 6.1. Schematic representation of the optical layer model used to evaluate the ellipsometry data. See text for details.

During the multiple reflection in the thin film of thickness, d_f, a phase shift (D) between each reflection in the film occurs. This is due to the differences in optical path for the light of wavelength, λ. The phase shift can be expressed as:

$$D = \frac{4\pi}{\lambda} d_f \sqrt{n_f^2 - n_1^2 \sin^2 \phi_1}$$ [6.8]

Here the wave length of the light, λ and the thickness of the film, d_f, have been introduced. The relation between the ellipsometer angles Δ and Ψ and the reflection coefficients now becomes:

$$\tan \Psi_f e^{i\Delta_f} = \rho_f = \frac{r_{pf}}{r_{sf}}$$ [6.9]

From equations [6.6] and [6.7] it is obvious that the reflection coefficients are now complex numbers. Hence, Δ, will vary with the thickness/refractive index of the film. A simple way to

illustrate the expected changes is to use the first order approximation for ρ_f expressed as the relative change when the thickness/refractive index of the film increases (Wang et al., 1990)

$$\frac{\partial \rho_f}{\rho_f} = \frac{\partial \tan \Psi_f}{\tan \Psi_f} + i \partial \Delta_f = \frac{i 4 \pi n_1 d_f \cos \phi_1}{\lambda} \cdot \frac{n_2^2 (n_f^2 - n_1^2)(n_2^2 - n_f^2)}{n_f^2 (n_2^2 - n_1^2)(n_2^2 \cot^2 \phi_1 - n_1^2)} \qquad [6.10]$$

From equation [6.10] it follows that for dielectric films and media; that is, when the refractive indices assume real values, the right hand side of equation [6.10] is the imaginary part of the left-hand side. From this it follows that the formation of a film will only be reflected in a change in Δ. Any measured changes in Ψ must depend on changes of the refractive index of the media or of the angle of incidence. The changes of angles of incidence can arise from changes in the curvature of the liquid interface. Such changes can occur due to changes in the wetting of the measuring cell and/or changes in the interfacial tension due to the adsorption of surface-active molecules. However, as Δ reflects the build up of the film essentially independently of Ψ, we can use changes in Ψ to correct for changes in the angle of incidence, e. g. due to changes in the curvature of the liquid interface. We also note from equation [6.10] that for $\phi_1 \sim 45°(\cot\phi_1 \sim 1)$, which is approximately the angle of incidence we have used, and for $n_1 > n_f > n_2$ we would expect an increase in Δ as the film is built up. On the other hand, if $n_f > n_1$ then a decrease in Δ from 180 degrees is expected. As the build up of film, for the system used, only changes one of the parameters, we can either determine the thickness and fix the refractive index or vice versa. Since the refractive index of the film determines whether Δ should increase or decrease, we chose to fix the thickness of the film and adjusted the refractive index of the film until the measured values of Δ were obtained. From the thickness and refractive index values we can obtain the amount of, for instance, a protein in the film (De Feijter et al., 1978; Cuypers et al., 1983). We used the formula derived by Cuypers et al. (1983), where the surface excess (or adsorbed amount), Γ, can be obtained as:

$$[6.11]$$

$$\Gamma = \frac{0.3 d_f (n_f^2 - n_{f0}^2)}{(n_f^2 + 2)[r(n_{f0}^2 + 2) - v(n_{f0}^2 - 1)]}$$

Here, r is the specific refractivity and v the partial specific volume of the adsorbing/deposited substance (e.g. protein) in the film and n_{f0} is the refractive index of the solvent in the film. In all the calculations, n_{f0} was set to be equal to the buffer value. For β-lactoglobulin an r value, obtained from the molar refractivity, of 0.249 ml g^{-1} and a v value of 0.749 mg ml^{-1} (Nylander and Wahlgren, 1994) were used. The corresponding values for sb-PC were set to r = 0.274 ml g^{-1} and v = 0.980 ml g^{-1} as reported by Kop et al. (1984). The expression for Δ and Ψ given in equation [6.9] is not complete for the optical system used in this study as depicted in Fig. 6.1. The ellipsometry data were recorded in air, which means that the light had to pass through the

air/oil and oil/air interfaces. We then have to introduce the transmission coefficients for these interfaces and arrive at the following expression for the measured Δ and Ψ:

$$\tan \Psi_{0f} e^{i\Delta_{0f}} = \frac{t_{p01} r_{pf} t_{p10}}{t_{s01} r_{sf} t_{s10}}$$

[6.12]

The refractive index data was taken from *Handbook of Chemistry of Physics* (1974). The value for the buffer was the water value of 1.3332, for caraway essential oil the value (1.4730) reported for one of its major constituents (limonene) was used and for the olive oil, the value for triolein (1.4621) was used. It should be noted these values were recalculated to $\lambda = 5320$ Å, from the reported values for sodium light $\lambda = 5893$ as described by Mahanty and Ninham (1974) and Böttcher and Bordewijk (1978). Hence the refractive indices used for $\lambda = 5320$ Å were 1.33494, 1.47569, and 1.46473 for buffer, caraway and olive oil, respectively. In the evaluation of the ellipsometry data, first the angle of incidence was determined by varying the ϕ_0 value until the experimental Ψ was obtained. In the next step we assumed a d_f-value, entered it in equation [6.12] and varied the refractive index of the film until the calculated value of Δ matched the experimentally obtained one. The calculations were done using Mathematica 3.0 (Wolfram Research, Champaign, IL) on a Macintosh Power PC. The d_f-value was chosen so that it matched the molecular dimensions of the adsorbing molecules; that is, for sb-PC half the thickness of a PC bilayer, ~18 Å (cf. the value reported by LeNeveu et al. (1976) for PC from egg yolk) and for β-lactoglobulin, 20 Å. The latter value is based on dynamic light scattering measurements of the thickness of adsorbed β-lactoglobulin layers (20 Å) on latex particles, reported by Dalgleish and Leaver (1991). Horne et al. (1998) and Mackie et al. (1991) reported layer thickness of β-lactoglobulin of 18 Å at the air/water interface from neutron reflectivity measurements and on polystyrene latex particles using X-ray scattering. This is slightly less than the diameter of the monomer, which is about 36 Å (Green and Aschaffenburg, 1959). It is reasonable to assume that the thickness of the layer should be a little less than the molecular dimensions, as one would expect that the protein is partly unfolded at the interface. Furthermore, the ellipsometer measures3 an average thickness, which is expected to be less than the protrusion of protein segments into the bulk solution (for a more detailed discussion see Kull et al., 1997).

RESULTS AND DISCUSSION

Emulsification experiments

Fig. 6.2 shows the average emulsion droplet diameter as a function of the sb-PC content in the two types of emulsions, one based on a triglyceride (olive oil) and the other based on hydrocarbon (caraway essential oil). With the emulsification procedure used, the average

emulsion droplet size was smaller in emulsions made with caraway essential oil than when olive oil was used as the dispersed phase. A difference between hydrocarbon (*n*-tetradecane) and triglyceride oil-in-water emulsions in systems containing mixtures of protein (β-casein) and sb-PC has also been reported by Courthaudon et al. (1991a). They found that much more lecithin is associated with *n*-tetradecane than with soy oil droplets. At low sb-PC/protein molar ratios, substantially more β-casein was adsorbed at the hydrocarbon/aqueous interface than at the triglyceride/aqueous interface.
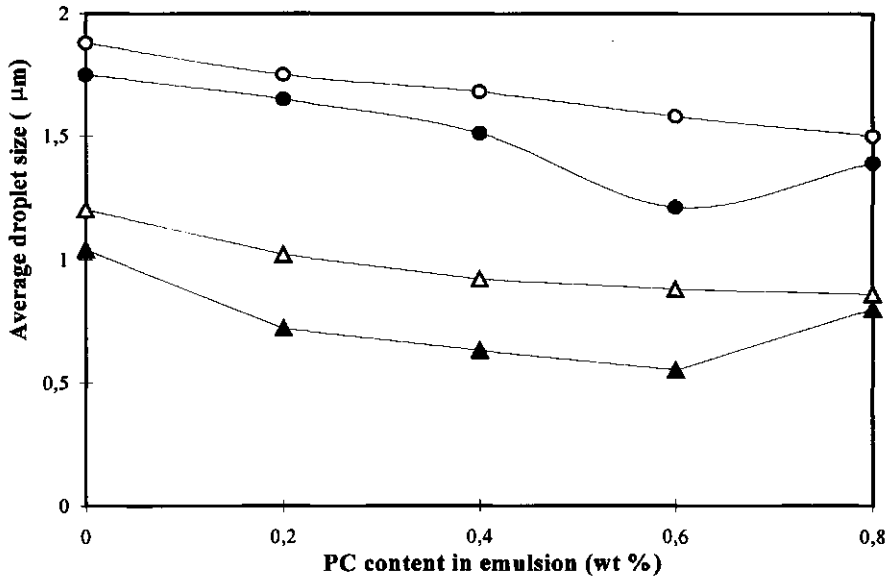
Fig. 6.2. Effect of sb-PC on droplet size of emulsions formed from olive and caraway oil emulsions stabilized by β-lg

—●— Olive oil, 2% b-lg;   —O— Olive oil,1% b-lg;
—▲— Caraway oil, 2% b-lg;   —△— Caraway oil,1% b-lg;

The reason for this difference is certainly associated with the much lower interfacial tension at the triglyceride/water interface than at the hydrocarbon/water interface (Fisher et al., 1985). The droplet size also decreases with increasing protein concentration as well as with increasing sb-PC content for both types of emulsions In the emulsions without sb-PC, an increased protein concentration from 1 to 2% leads to a reduction in the average particle size by 6.9 and 13% in olive and caraway oil emulsions, respectively. The addition of sb-PC leads to an even larger decrease in the droplet size of 20–47%, depending on the oil, protein concentration and sb-PC content. It is clear that the effect of sb-PC is more pronounced in the emulsions with the higher protein concentration (2%). Here it is important to remember that a

decrease in emulsion droplet size means an increase in total surface area of the oil/aqueous interface. For instance a decrease in the radius of 20% gives a surface area which is 1.25 times larger and a 50% reduction gives twice the area to cover. This means that more molecules are needed in order to form a stable emulsion. Since we observed a reduction in droplet size as an effect of an increase in protein concentration, we can be confident that a mixed protein/lipid film is formed at the oil/aqueous interface. It is also obvious from the protein adsorption data shown in Table 6.1, that sb-PC is unable to replace the protein completely. This is in agreement with earlier studies, where lecithin was found to be less efficient than other surfactants in displacing milk proteins from the oil/water interface (Courthaudon et al., 1991a; Dickinson and Iveson, 1993). Even at high phospholipid:protein ratios, protein and sb-PC coexist at the interface (Courthaudon et al., 1991b; Fang and Dalgleish, 1993). If we again consider the adsorption data in Table 6.1, we observe that the maximum reduction in the adsorbed amount is by a factor of three for the caraway oil. We know that the average diameter decreases up to a maximum of about 50%. This corresponds to an area increase by a factor of two as mentioned above.

Table 6.1. Adsorbed amount of protein at the caraway essential oil (EO)/water and olive oil (OO)/water interfaces in emulsions stabilised by 1 and 2% β-lactoglobulin (BLG). The data obtained from measurements of the depletion of protein in solution and droplet size measurements are given as a function of sb-PC content in the oil.

sb-PC content (%) in emulsions	OO emulsions $(mg\ g^{-1})^1$		EO emulsions $(mg\ g^{-1})$	
	1 % BLG	2 % BLG	1 % BLG	2 % BLG
0.0	28.24 (7.91)	32.42 (8.44)	37.77 (5.04)	46.57 (6.45)
0.2	25.33 (6.59)	27.35 (6.72)	29.06 (3.48)	32.23 (3.18)
0.4	22.20 (5.55)	25.20 (5.66)	25.07 (2.74)	29.13 (2.45)
0.6	16.33 (3.53)	19.13 (3.44)	20.66 (2.06)	21.88 (1.54)
0.8	15.88 (3.54)	18.60 (3.85)	17.33 (1.82)	20.83 (2.22)

[1]Adsorbed amount of β-lactoglobulin is expressed in mg for 1g of dispersed phase (oil). The values in parentheses are given in mg m^{-2} based on area calculated from droplet size.

Thus we can conclude that sb-PC is likely to replace some of the protein at the oil/aqueous interface. It is interesting to note that a minimum particle average diameter is reached at 0.6% sb-PC for both types of oil. One might speculate that at this particular protein/lipid ratio the mixed layer is able to cover the largest surface area at the oil/aqueous interface; and thus there is enough surface-active material to form these small droplets.

Figures 6.3–6.6 show the effects of sb-PC and β-lactoglobulin on the stability of caraway and olive oil emulsions in terms of cream layer thickness. It is clear that the stability of oil-in-water emulsions is strongly dependent on protein and phospholipid concentrations. In general, emulsions with triglyceride oil were shown to be more stable than those made with caraway

essential oil as the dispersed phase. However, the stability of the emulsions can be improved considerably by adding sb-PC. This effect is substantially larger for caraway oil than for olive oil. In fact, the cream layer from caraway oil after 5 days at the highest sb-PC content was substantially thinner than that formed from olive oil. If we look at the different oils separately, we see that the increase in emulsion stability follows the decrease in emulsion droplet size, which is what one would expect. On the other hand, if we compare caraway oil emulsions with those formed from olive oil, the olive oil emulsions seem to be more stable in spite of their larger droplet size. As discussed above, this is probably partly due to the lower interfacial tension of the triglyceride/aqueous interface compared with the caraway oil/aqueous interfacial tension. Another effect might be differences in surface coverage. The higher total surface area of the caraway oil emulsions might not be completely covered with lipids or proteins, which in turn would lead to an increased coalescence of the emulsion droplets. Judging from the adsorption data presented in Table 6.1, almost 50% of the available protein from a 1% solution was adsorbed at the droplet surface of the caraway oil emulsion in the absence of sb-PC. In the emulsions containing 2% β-lactoglobulin, only about 30% of added protein is depleted from the solution during emulsification. We also observed a greater stability for both oils, which is further increased if sb-PC is added. Thus, one might speculate that the gaps in the coverage of protein are filled in with sb-PC. The only aspect which does not fit in with this view is the high protein surface load we recorded, and this will be discussed elsewhere.

The properties of the oil/water interface were further investigated by *in situ* ellipsometry and the experimental data are summarised in Table 6.2. The values given here were measured 1 h after adding the oil (with or without sb-PC) or injecting the protein. The measured Δ-values for both the olive oil and the caraway oil/aqueous interfaces were around 180°. This means that no layer is present between the oil/water interface or if a layer is present it must have the same refractive index as either the water or the oil. It should be noted that very pure oils were used. When sb-PC is added to the oil we see marked changes in the Δ-values. If we first consider the caraway/sb-PC mixture, the amount adsorbed seems to be independent of the sb-PC concentration used within the given concentration range (1–5% in the oil). No time dependence of the adsorption could be detected. The value ~1.4 mg m^{-2} compares well with
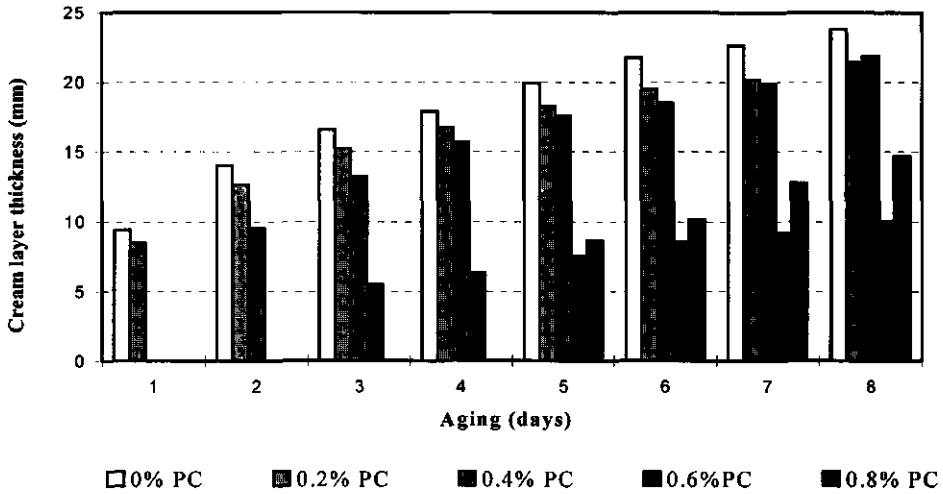
Fig. 6.3. Emulsion stability shown as the cream layer thickness against time. The emulsions were prepared from 15wt% olive oil and total β-lg concentration of 1wt%.
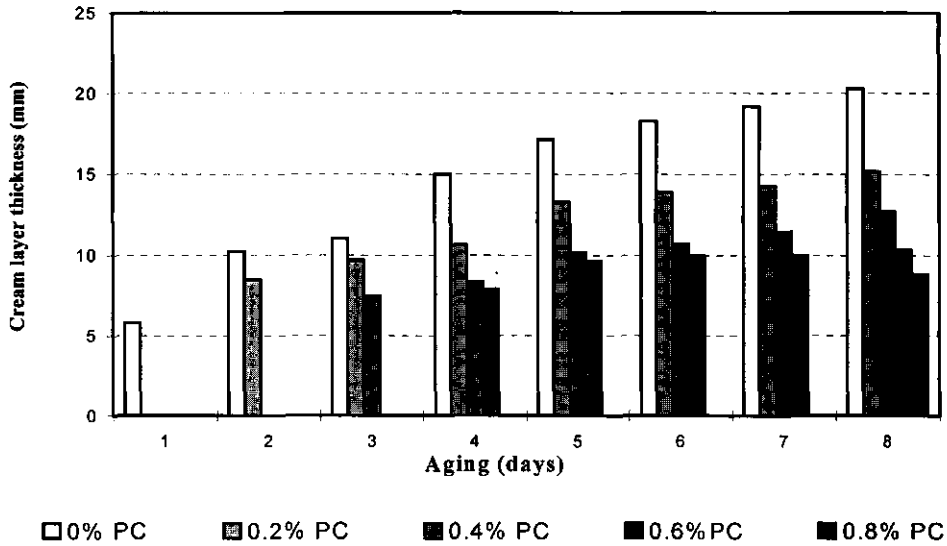
Fig. 6.4. Emulsion stability shown as the cream layer thickness against time. The emulsions were prepared from 15wt% olive oil and total β-lg concentration of 2 wt%
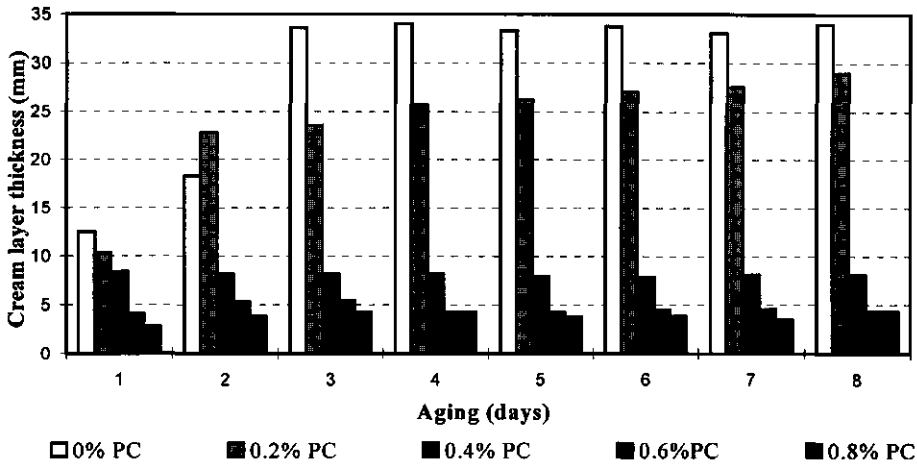
Fig. 6. 5. Emulsion stability shown as the cream layer thickness against time. The emulsions were prepared from 15wt% caraway oil and total β-lg concentration of 1wt%.
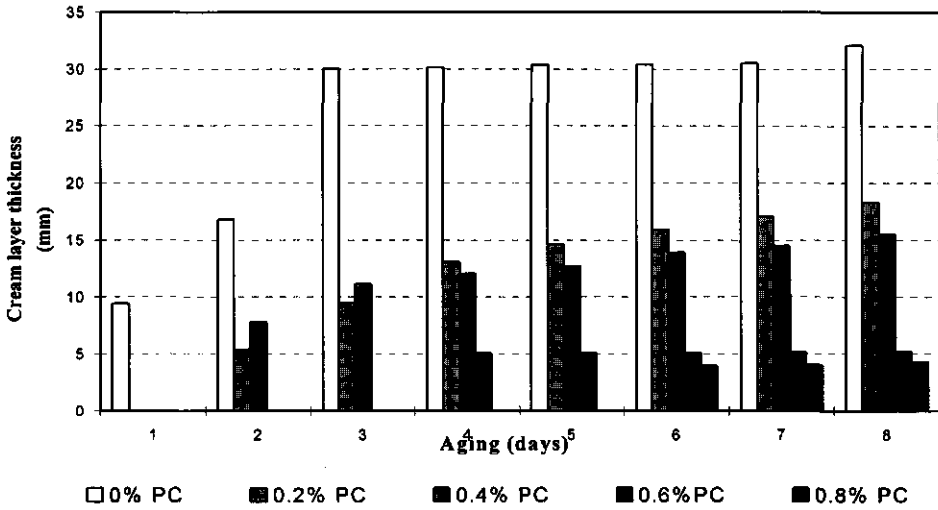
Fig 6.6. Emulsion stability shown as the cream layer thickness against time. The emulsions were prepared from 15wt% caraway oil and total β-lg concentration of 2wt%.

half the value of 4.0 mg m^{-2} reported for deposited dioleoylphosphatidylcholine (DOPC)/dioleoylphosphatidylserine (DOPS) bilayers (Kop et al., 1984). In other words, close to monolayer coverage of sb-PC at the oil/water interface was reached. We also note that the refractive index of this film has a lower value than that of the oil as we would expect from a Δ-value > 180°. This accounts for the uptake of water by the sb-PC polar head group. For the olive oil and sb-PC mixtures, a completely different picture arises; in fact we could only investigate the oil/water interfaces of these mixtures at concentrations of 0.1% and below with the experimental procedure that we used. At higher sb-PC concentrations, the oil and water start to mix and no defined interface is formed. This is again related to the lower interfacial tension of the triglyceride/aqueous interface.

In the emulsion experiments it was also found that the emulsions formed with the triglyceride oil were more stable. We can get an indication of this behaviour if we look at the values for mixtures containing 0.01% of sb-PC. Although the adsorbed amounts are almost the same and no time dependence could be measured, the refractive index of the film is lower than for the sb-PC layer at the caraway oil/aqueous interface. In fact we could not fit our data unless we increased our values of the thickness from 18 to 22 Å. This, together with the lower refractive index, suggests that the sb-PC film at the olive oil/aqueous interface is more hydrated than at the caraway/aqueous interface. If we increase the sb-PC concentration in the olive oil to 0.1%, this is even more evident. We do not arrive at a plateau but the Δ value increases exponentially with time. If we assume that the refractive index is constant and the same as for the monolayer, we can calculate the increase in thickness. After 1 h we arrive at a mean thickness of about 53 Å, which is more than an sb-PC bilayer. The amount adsorbed is also about 2.5 times that observed at an sb-PC concentration of 0.01%. However, it is probably unlikely that the sb-PC layer maintains a well ordered lamellar structure. We can conclude that there are marked differences in the properties of the caraway and olive oil aqueous interfaces. It should also be noted that sb-PC was much more easy to dissolve in the caraway oil than in the olive oil. The lower solubility of sb-PC in the olive oil can in fact drive the lipid to the aqueous interface, where it can eventually form a liquid crystalline phase.

The adsorption of β-lactoglobulin onto the oil/aqueous interface led to a decrease in Δ, which in turn indicates a refractive index of the film larger than the oil. Thus, the layer of β-lactoglobulin should be quite dense. This is also in agreement with neutron reflectivity data of β-lactoglobulin adsorption at the air/aqueous interface (Atkinson et al., 1995). They fitted data to a two-layer model, which had with an inner layer 10 Å thick with a protein volume fraction of 0.97 and an outer layer 20 Å thick with a protein volume fraction of 0.13. From these values, they calculated an adsorbed amount of 1.69 mg m^{-2}, which is close to the values we found (Table 6.2). It is also noteworthy that we obtained almost the same adsorbed amount of

Table 6.2. Ellipsometry data for the adsorption of soyabean phosphatidylcholine (sb-PC) and β-lactoglobulin (BLG) at the oil/aqueous interface. The refractive index of the oil (n_1) was 1.47569 for the caraway oil essential oil (EO) and 1.46472 for the olive oil (OO) at $\lambda = 5320\text{Å}$. The corresponding value for the aqueous phase, $n_2 = 1.33494$.

Substance	Δ	Ψ	ϕ_0	ϕ_1	n_f	d_f[1] (Å)	Γ_f[2] (mg m^{-2})	Δ_1[3]	Ψ_1[3]
OO	180.05	7.44	69.594	39.783	–	–	–	180.00	5.615
OO	179.95	7.44	69.594	39.783	–	–	–	180.00	5.615
OO + sb-PC (0.01%)	180.54	7.47	69.555	39.771	1.4251	22[4]	1.51	180.54	5.641
OO + sb-PC (0.1%)	181.30[5]	7.44[5]	69.552	39.770	1.4251[6]	52.9[6]	3.65	181.30	5.641
OO + BLG (0.1%)	179.75	7.49	69.532	39.764	1.4779	20	1.62	179.75	5.658
OO + BLG (0.2%)	179.80	7.50	69.519	39.760	1.4755	20	1.60	179.80	5.666
OO + BLG (0.2%)	179.70	7.55	69.455	39.740	1.4805	20	1.65	179.70	5.709
EO	180.05	5.60	72.762	40.332	–	–	–	180.00	3.996
EO	180.00	5.45	72.986	40.390	–	–	–	180.00	3.867
EO + sb-PC (1.3%)	180.60	5.70	72.623	40.295	1.4400	18	1.43	180.60	4.076
EO + sb-PC (2.6%)	180.60	5.90	72.346	40.221	1.4378	18	1.44	180.60	4.239
EO + sb-PC (3.8%)	180.60	5.75	72.554	40.260	1.4395	18	1.43	180.60	4.117
EO + sb-PC (5.1%)	180.65	5.55	72.832	40.350	1.4374	18	1.40	180.65	3.955
EO + sb-PC (5.1%)	180.60	5.75	72.554	40.260	1.4395	18	1.44	180.60	4.117
EO + BLG (0.5%)	179.85	5.48	72.930	40.375	1.4757	20	1.66	179.85	3.899
EO + BLG (1.0%)	179.85	5.61	72.749	40.328	1.4815	20	1.66	179.85	4.004

[1]Assumed value based on half the thickness of egg yolk PC bilayer (LeNeveu et al., 1976) and for BLG the thickness value from light scattering studies (Dalgleish and Leaver, 1991).

[2]For the calculation of surface excess, Γ, according to equation [6.11] the values of r and v for β-lactoglobulin, were 0.249 ml g^{-1} and v = 0.749 ml g^{-1} (Nylander and Wahlgren, 1994) and the corresponding values for sb-PC, were 0.274 ml g^{-1} and 0.980 ml g^{-1} (Kop et al., 1984), respectively.

[3]These values were determined as measured from the oil phase, that is as determined from equation [6.9], using the same values for n_f and d_f.

[4]No solution was found unless the thickness was increased to 22 Å.

[5]The Δ-value appeared to increase exponentially. The value after 1h is given.

[6]As the adsorption seemed to increase beyond monolayer coverage, the refractive index was fixed and the thickness was varied.

β-lactoglobulin at the olive oil/aqueous as at the caraway oil/aqueous interface. There was also no dependence of the protein concentration within the limited protein concentration range investigated (from 0.1 to 1%). Elofsson et al (1997) determined the adsorption isotherms for the β-lactoglobulin variants A and B on hydrophobised silica surfaces using ellipsometry. They found large differences in the adsorption isotherms, which could be directly correlated with the self-association of the protein. However, at about 0.1% a plateau in the adsorption isotherms was reached for both genetic variants, where both gave an adsorbed amount of about 2.5 mg m^{-2}. This is only slightly higher than that observed for the air/water and oil/water interfaces, but larger conformational changes are expected to occur when the protein adsorbs at a liquid interface rather than a solid surface. In this case, the area per molecule will be larger and the amount adsorbed will be less.

The protein surface concentrations on both types of droplet surface, as determined from solution depletion measurements, are substantially higher (Table 6.1). In olive oil emulsions, surface concentrations of 7.91 and 8.44 mg m^{-2} were recorded for 1 and 2% β-lactoglobulin, respectively. For the caraway oil emulsions, these values were lower (5.04 and 6.45 mg m^{-2}, respectively), due to smaller droplet sizes and thus higher surface area created during the homogenisation. Dickinson et al. (1989) reported values of 3.0 mg m^{-2} for β-lactoglobulin surface coverage on *n*-tetradecane emulsion droplets. However, they used a larger amount of oil (20%) and a lower protein concentration (0.5%). It should be noted that determining the absolute surface excess by the solution depletion method is enough complicated thing and our purpose was mainly to record the trends. When separating the oil from the serum, proportionally more of the protein might be associated with the oil droplets, thus leading to an overestimation of the amount adsorbed. One way to overcome this problem is to wash the separated cream and determine its protein concentration. However, the risk then is that some of the protein might desorb. The other source of error is the determination of droplet size. With the method used we can only measure droplets in the μm-scale. Any smaller droplets, which will contribute proportionally more to the surface area than larger particles of the same total volume, are not taken into account. Thus it is likely that we will underestimate the total surface area and hence overestimate the amount adsorbed. It is also possible that some of the protein will denature and precipitate out from the solution during the harsh mechanical treatment involved in the emulsification process. This will also lead to an overestimation of the amount adsorbed. The fact that our ellipsometry data indicate that the same amount of β-lactoglobulin is adsorbed on the olive oil/aqueous as on the caraway oil/aqueous interface does not necessarily contradict the results obtained from the solution depletion measurements. The significantly larger protein surface excess determined for the olive oil/aqueous interface may have to do with differences in surface-to-volume ratio. In the ellipsometry experiment, we found a constant surface area-to-oil volume ratio of 20 cm^2:10 cm^3 (or about 2 cm^{-1}). The

corresponding value for the olive oil/aqueous emulsion is $4\pi(1.75 \ 10^{-4})^2/((4/3)\pi(1.75 \ 10^{-4})^3) = 3/1.75 \ 10^{-4} \sim 1.7 \ 10^4$ cm^{-1} and for the caraway oil/aqueous emulsion it is about $2.9 \ 10^4$ cm^{-1}. Thus, in the ellipsometry experiments, the bulk solution has to supply an area about 9000–15000 times smaller than in the emulsion experiments. The ellipsometry measurements show that β-lactoglobulin has the same affinity to the two types of oil interfaces, provided that it is not limited by the amount of available protein. The larger amount adsorbed obtained from the solution depletion measurements on the olive oil/aqueous interface compared with the caraway oil/aqueous interface correlates with the large surface area-to-volume ratio of the latter interface. As suggested above, the adsorption at the caraway oil/aqueous interface is limited by the amount of protein available relative the adsorption at the olive oil/aqueous interface.

Finally we did some measurements of the β-lactoglobulin adsorption at the interface between buffer and the caraway oil containing sb-PC (data not shown). Here the presence of the protein did not have any effect on the ellipsometry angles. This means that the protein does not become adsorbed to the sb-PC coated oil/water interface. Malmsten (1994) reported that the adsorption of serum proteins is generally significantly less on a phosphatidylcholine-coated solid surface compared with a hydrophobic surface. It must also be borne in mind that ellipsometry only measures the effective amount at the interfaces. Thus, the composition of the interface layer might change (e. g. a mixed protein layer may be formed), while the total amount adsorbed is the same. The solution depletion measurements of the amount adsorbed on emulsion droplets suggest that some protein remains at the interface even at the highest sb-PC concentration. We again stress that the surface area in the emulsification experiments was several orders of magnitude larger.

ACKNOWLEDGEMENTS

REFERENCES

Atkinson, P.J.; Dickinson, E.; Horne, D.S.; Richardson, R.M. Neutron reflectivity of adsorbed β-casein and β-lactoglobulin at the air/water interface. *J. Chem. Soc. Faraday Trans.* **1995**, 91, 2847-2854.

Azzam, R.M.A.; Bashara, N.M. *Ellipsometry and Polarized Light.* North Holland Elsevier Science Publishers, Amsterdam, 1987.

Bergenstahl, B.A.; Claesson, P.M. Surface forces in emulsions. In *Food Emulsions. 3rd ed.*, Eds. Larsson, K.; Friberg, S.E.; Marcel Dekker, New York, NY, 1997; pp57-109.

Brownlow, S.; Morais Cabral, J.H.; Cooper, R.; Flower, D.R.; Yewdall, S.J.; Polikarpov, I.; North, A.C.T.; Sawyer, L. Bovine β-lactoglobulin at 1.8 Å resolution - still an enigmatic lipocalin. *Structure*, **1997**, 5, 481-495.

Böttcher, C.J.F.; Bordewijk, P. *Theory of electric polarization. Volume 2. Time-dependent fields*, Elsevier, Amsterdam, 1978.

Chen, J.; Dickinson, E Time-dependent competitive adsorption of milk proteins and surfactants in oil-in-water emulsions. *J. Sci. Food Agric.* **1993**, 62, 283-289.

Courthaudon, J.L.; Dickinson, E.; Christie, W.W. Competitive adsorption of lecithin and β-casein in oil-in-water emulsions. *J. Agric. Food Chem.* **1991a**,. 39, 1365-1368.

Courthaudon, J.L.; Dickinson, E.; Dalgleish, D.G. Competitive adsorption of β-casein and nonionic surfactants in oil-in-water emulsions. *J. Coll. Int. Sci.* **1991b**, 145, 390-395.

Courthaudon, J.L.; Dickinson, E.; Matsumura, Y.; Williams, A. Influence of emulsifier on the competitive adsorption of whey proteins in emulsions. *Food Struct.* **1991c**, 10, 109-115.

Courthaudon, J.L.; Dickinson, E.; Matsumura, Y.; Clark, D.C. Competitive adsorption of β-lactoglobulin + Tween 20 at the oil- water interface. *Colloids Surf.* **1991d**, 56, 293-300.

Cuypers, P. A.; Corsel, J. W.; Janssen, M. P.; Kop, J. M. M.; Hermens, W. Th.; Hemker, H. C. The adsorption of prothrombin to phosphatidylserine multilayers quantitated by ellipsometry. *J. Biol. Chem.* **1983**, 258, 2426-2431.

Dalgleish, D.G.; Euston, S.E.; Hunt, J.A.; Dickinson, E. Competitive adsorption of _-lactoglobulin in mixed protein emulsions. In: *Food polymers, gels and colloids*; ed. E.Dickinson. Royal Society of Chemistry, Cambridge, 1991; pp 485-489.

Dagleish, D. G.; Leaver, J. Dimensions and possible structures of milk proteins at oil-water interfaces. In *Food Polymers, Gels and Colloids*, ed. E. Dickinson. Royal Society of Chemistry, Cambridge, UK, 1991; pp 113-122.

De Feijter, J.A.; Benjamins, J.; Veer, F. A. Ellipsometry as a tool to study the adsorption behaviour of synthetic and bioplymers at the air-water interface. *Biopolymers* **1978**, 17, 1759-1772.

De Wit, J.N. Functional properties of whey proteins. *In Developments in Food Chemistry 4*, ed. P.F. Fox. Elsevier Applied Science, London, 1989, pp 285-321.

Dickinson, E.; Owusu, R.K.; Sze Tan; Williams, A. Oil - soluble surfactants have little effect on competitive adsorption of α-lactalbumin and β-lactoglobulin in emulsions. *J. Food Sci.* **1993**, 58, 295-598.

Dickinson, E.; Iveson, G. Adsorbed films of β-lactoglobulin + lecithin at the hydrocarbon-water and triglyceride - water interfaces. *Food Hydrocolloids*, **1993**, 6, 533-541.

Dickinson, E.; Tanai, S. Protein displacement from the emulsion droplet surface by oil-soluble and water soluble surfactants". *J. Agric. Food Chem.* **1992,** 40,179-183.

Dziezak, J.D. Microencapsulation and encapsulated ingredients. *Food Techn.* **1988**, 42, 136-151.

Elofsson, U. M.; Paulsson, M. A.; Arnebrant, T. Adsorption of β-lactoglobulin A and B in relation to self-association: Effect of concentration and pH. *Langmuir* **1997**, 13, 1695-1700.

Faldt, P.; Bergenstahl, B. Spray-dried whey protein/lactose/soybean oil emulsions. 1. Surface composition and particle structure. *Food Hydrocolloids*, **1996a**, 10, 421-429.

Faldt, P.; Bergenstahl, B. Spray-dried whey protein/lactose/soybean oil emulsions. 2.Redispersibility, wettability and particle structure. *Food Hydrocolloids*, **1996b**, 10, 431-439.

Fang, Y.; Dalgleish, D.G. Casein adsorption on the surfaces of oil-in-water emulsions modified by lecithin. *Coll. Surf. B: Biointerfaces*, **1993**, 1, 357-364.

Fisher, L.R.; Mitchell, E.E.; Parker, N.S. Interfacial tensions of commercial vegetable oils with water. *J. Food Sc.* **1985**, 50, 1201-1202.

Fligner, K.L.; Fligner, M.A.; Mangino, M.E. The effects of compositional factors on the short-term physical stability of a concentrated infant formula. *Food Hydrocolloids*, **1990,** 4, 95-104.

Green, D.W.; Aschaffenburg, R. Two fold symmetry of the β-lactoglobulin molecule in crystals. *J. Molec. Biol.* **1959**, 1, 54-64.

Handbook of Chemistry and Physics, CRC Press, Cleveland, 1974.

Harke, M.; Teppner, R.; Schulz, O.; Motschmann, H.; Orendi, H. Description of a single modular optical setup for ellipsometry, surface plasmons, waveguide modes, and their corresponding imaging techniques including Brewster angle microscopy. *Review of Scientific Instruments* **1997**, 68, 3130-3134.

Horne, D.S.; Atkinson, P J.; Dickinson, E.; Pinfield, V.J.; Richardson, R. M. Neutron reflectivity study of competitive adsorption of β-lactoglobulin and nonionic surfactant at the air-water interface. *Int. Dairy J.* **1998**, 8, 73-77.

Jackson, L. S.; Lee, K. Microencapsulation in the food industry. *Lebensm. – Wiss. Technol.* **1991**, 24, 289-297.

Kim,Y.D.; Morr, C.V.; Schenz, T.W. Microencapsulation properties of gum arabic and several food proteins: liquid orange oil emulsion particles. *J. Agric.Food Chem.* **1996**, 44, 1308-1313.

Kim,Y.D.; Morr, C.V. Microencapsulation properties of gum arabic and several food proteins: spray-dried orange oil emulsion particles. *J. Agric.Food Chem.* **1996**, 44, 1314-1320.

Kinsella, J.E. Milk proteins: physicochemical and functional properties. *Crit. Rev. Food Sci. Nutr.* **1990,** 21,197-262.

Kinsella, J.E.; Whitehead, D.M. Proteins in whey: chemical, physical and functional properties. *Adv. Food Nutr.Res.* **1989**, 33, 343-438.

Kinsella, J.E.; Whitehead, D.M.; Brady J.; Bringe N.A. Milk proteins: possible relationships of structure and function. In *Developments in Food Chemistry 4. Functional Milk proteins*; ed. P.F. Fox, Elsevier Applied Science Publishers: London, 1989; pp 55-95.

Kop, J.M.M.; Cuypers, P.A.; Lindhout, T.; Hemker, H. C.; Hermens, W.Th. The adsorption of prothrombin to phospholipid monolayers quantitated by ellipsometry. *The J. Biol. Chem.,* **1984**, 259, 13993-13998.

Kull, T.; Nylander, K.; Tiberg, F.; Wahlgren, N.M. Effect of surface properties and added electrolyte on the structure of β-casein layers adsorbed at the solid/aqueous interface. *Langmuir* **1997,** 13, 5141-5147.

Kurihara, K.; Katsuragi, Y. Specific inhibitor for bitter taste. *Nature,* **1993,** 365, 213-214.

LeNeveu, D.M.; Rand, R.P.; Parsegian, A.V. Measurements of forces between lecithin bilayers. *Nature* **1976,** 259, 601-603.

Lowry, O.H.; Rosebrough, N.J.; Farr, A.L.; Randall, R.J. Protein measurement with the folin phenol reagent. *The J.Biol. Chem.* **1951**, 193, 265-275.

Mackie, A.K.; Mingins, J.; Dann, R.; North, A.N. Preliminary studies of β-lactoglobulin adsorbed on polystyrene latex. In *Food Polymers, Gels and Colloids*, ed. E. Dickinson. Royal Society of Chemistry, Cambridge, UK, 1991; pp 96-112.

Mahanty, J.; Ninham, B. W. *Dispersion forces.* Academic Press, London, 1976.

Malmsten, M. Ellipsometry studies of protein adsorption at lipid surfaces. *J.Colloid Interf. Sci.,* **1994,** 168, 247-254.

McCrackin, F.L.; Passaglia, E.; Stromberg, R.R.; Steinberg, H.L. Measurements of the thickness and refractive index of very thin films and the optical properties of surfaces by ellipsometry. *J.Res.National Bureau Stand. – A. Phys.Chem.* **1963**, 67A, 363-377.

Moreau, D.L.; Rosenberg, M. Oxidative stability of anhydrous milkfat microencapsulated in whey proteins. *J.Food Sci.* **1996**, 61, 39-43.

Morr, C.V. Functional properties of milk proteins an their use as food ingredients. In *Developments in Dairy Chemistry*, ed. P.F.Fox. Applied Sci., London, United Kingdom, 1982; p.375

Morr, C.V.; Ha, E.Y.W. Whey protein concentrates and isolates: processing and functional properties. *CRC Crit.Rev. Food Sci. Nutr.*, **1993**, 33, 431-476.

Nylander, T.; Wahlgren, N. M. Competitive and sequential adsorption of β-casein and β-lactoglobulin on hydrophobic surfaces and the interfacial structure of β-casein. *J.Colloid Interf. Sci.* **1994**, 162, 151-162.

Pearce, K.N.; Kinsella, J.E. Emulsifying properties of proteins: evaluation of a turbidimetric technique. *J. Agric. Food Chem.* **1978**, 26, 716-723.

Rish, S.J.; Reineccius, G.A. Spray-dried orange oil: effect of emulsion size on flavor retention and shelf stability. In *Flavor encapsulation*. eds.. Rish, S.J.; Reineccius, G.A.; ACS symposium series 370; American Chemical Society: Washington, D.C., 1988; pp 67-77.

Rosenberg, M.; Young, S.L. Whey proteins as microencapsulating agents. Microencapsulation of anhydrous milkfat-structure evaluation. *Food Struct.* **1993**, 12, 31-41.

Sawyer, L.; Brownlow, S.; Polikarpov, I.; Wu, S.-Y. β-Lactoglobulin: Structural studies, biological clues. *Int. Dairy J.* **1998**, 8, 65-72.

Sheu, T.Y.; Rosenberg, M. Microencapsulation of volatiles by whey proteins. *J.Dairy Sci.* **1993**, 76 (Suppl.1), 28.

Shimizu, M.; Saito, M.; Yamauchi, K. Emulsifying and structural properties of β-lactoglobulin at different pHs. *Agric. Biol. Chem.* **1985**, 49, 189-194.

Shinoda, K.; Araki, M.; Sadaghiani, A.; Khan, A.; Lindman, B. Lecithin-based microemulsions: Phase behavior and microstructure. *J.Physical Chem.* **1991**, 95, 989-993.

Tomas, A.; Courthaudon, J.C.; Paquet, D.; Lorient, D. Effect of surfactant on some physico-chemical properties of dairy oil-in-water emulsions. *Food Hydrocolloids*, **1994**, 8, 543-553.

Walstra, P. The role of proteins in the stabilisation of emulsions. In *Gums and Stabilisers for the Food Industry*. Eds. G.O.Phillips, P.A.Williams. IRL Press: Washington, DC, 1988; pp 323-336.

Wang, J.; Andrade, J.D.; Lin, J.; Christensen, D.A. Improved sensitivity in ellipsometry of thin biochemical films by employing sublayers. In *Modeling of Optical Thin Films II*, ed. M. R. Jacobson. *Proceedings of SPIE--The International Society for Optical Engineering* 1990; pp 72-78.

Yamamoto, Y.; Araki, M. Effects of lecithin addition in oil or water phase on the stability of emulsions made with whey proteins. *Bioscience Biotechn. Biochem.* **1997**, 61, 1791-1795.

PROPERTIES OF CARAWAY ESSENTIAL OIL ENCAPSULATED INTO MILK PROTEIN-BASED MATRICES

This chapter is based on the following articles/manuscripts:

1. Venskutonis, R.; Bylaitė, E.; Narkevičius, R. Optimisation of encapsulation conditions of caraway (*Carum carvi* L.) essential oil. In Proceedings of the 2nd main meeting of EC Copernicus Concerted Action *"Process Optimisation and Minimal Processing of Foods"* **1996**, Vol. 3 *"Drying"*, ed. J.C. Oliveira, pp 95–101.

2. Bylaitė, E.; Venskutonis, R.; Kvietkauskaitė, D. Encapsulation of caraway (*Carum carvi* L.) essential oil in different matrices. In Proceedings of the International Workshop on Bioencapsulation VI *"From Fundamentals to Industrial Applications"*, eds. F.Godia, D.Poncelet, Universitat Autonoma de Barcelona, talk 5.1, **1997**.

3. Bylaitė, E.; Venskutonis, R. Stability of microencapsulated caraway (*Carum carvi* L.) essential oil during storage. In Proceedings of the 3rd main meeting of EC Copernicus Concerted Action *"Process Optimisation and Minimal Processing of Foods"*, Vol. 3 *"Drying"*, eds. J.C. Oliveira and F.A.R. Oliveira, **1997**, pp 81–87.

4. Bylaitė, E.; Venskutonis, R.; Maždžierienė, R. Properties of encapsulated caraway essential oil into milk proteins based matrices. Submitted to *J. Agric. Food Chem.*

ABSTRACT

The encapsulating properties of whey protein concentrate (WPC), skimmed milk powder (SMP) and their mixtures with maltodextrines (MD) for the encapsulation of caraway essential oil by spray drying were evaluated. Encapsulation efficiency (EE) was higher in WPC-based than in SMP-based matrices. Partial replacement of WPC by different MD increased the retention of volatiles during spray drying and enhanced the protective properties of solidified capsules to oxidation and the release of volatiles during storage. The opposite tendency was shown by SMP matrices: adding MD to the wall composition resulted in a lower retention of volatiles during drying and lower stability of oxidation compared with sole SMP and all WPC matrices. Dynamic headspace analysis (DHS) was applied to determine the release rate of volatiles from the microencapsulated caraway oil particles. The results revealed that combined matrices of SMP and carbohydrates had the highest volatile release rate. Partial replacement of WPC by MD significantly reduced the release of volatiles from capsules as determined by DHS. The flavour profile of caraway oil entrapped in the matrices was similar to that in pure essential oil: a slight decrease in limonene content was recorded in some matrices. Scanning electron microscopy (SEM) results for microencapsulated particles showed WPC-based matrices to have fewer visible cracks and holes compared with SMP-based matrices. More dented surfaces could be observed on particles with MD as compared to sole WPC.

It was concluded that WPC-based matrices were more effective as caraway oil encpsulating agents than those using SMP. On the basis of the results for EE, DHS, and stability against oxidation, it was shown that the incorporation of carbohydrates into WPC results in the production of more effective microencapsulants.

INTRODUCTION

Flavour compounds are rather volatile liquids and generally thermally or chemically labile in native conditions. Microencapsulation has become an attractive approach to converting liquid food flavourings into a dry, free-flowing powder form which is easy to handle and incorporate into a dry food system (Jackson and Lee, 1991). The process is defined as a physical one where thin films or polymer coats are applied to small solid particles, droplets of liquids or

gases (Bakan, 1973). Besides the change in the physical characteristics of the original material, food flavours are encapsulated for several other reasons: (1) to retain them in a food product during storage; (2) to protect the flavour from undesirable interactions with the food; (3) to minimise flavour/flavour interactions within a mixture; (4) to guard against light, heat, moisture or air induced reactions or oxidation; (5) to deliver the controlled or delayed release of flavour; and (6) to mask objectionable flavours (Reineccius, 1991). Microencapsulation can be accomplished by different techniques: spray drying, spray chilling and spray cooling, extrusion, air suspension coating, multi-orifice centrifugal extrusion, coacervation/phase separations, liposome entrapment, inclusion complexion, co-crystallisation, and interfacial polymerisation (Dziezak, 1988; Jackson and Lee, 1991). However, spray drying remains the preferred method for encapsulating flavours due to its low cost and readily available equipment, although there are disadvantages to this method, e.g. the loss of volatiles, degradation of sensitive compounds and the need for further fine powder processing such as agglomeration to instantise the dried material or make it more readily soluble if it is for a liquid application (Reineccius, 1988; Rish, 1995). Leaflash spray drying modification has been proposed in which the drying air is at very high temperature (300–400°C) and flows at a very high velocity (Bhandari et al., 1992). It was found that citral and linalyl acetate could be spray dried with little impact on the compounds themselves.

Different types of coating materials have been used to produce microcapsules, including gums, carbohydrates, celluloses, lipids, inorganic materials and proteins. However, carbohydrate-based matrices (gums, maltodextrins, modified starch, and corn syrup solids) predominate in flavour encapsulation (Beatus et al., 1985; Chang et al., 1988; Rish and Reineccius, 1988; Trubiano and Lacourse, 1988; Bangs and Reineccius, 1990; Rosenberg et al., 1990; Reineccius, 1991; Bhandari et al., 1992, 1998, 1999; Thevenet, 1995; Kollengode and Hanna, 1997; Chattopadhyayja et al., 1998; Che Man et al., 1999; Zeller et al., 1999). The main disadvantage of most carbohydrate coating materials is their low emulsifying capacity and marginal retention of volatiles. Various functional properties of food proteins, i.e. solubility, viscosity, emulsification, film formation, the ability to interact with water, small ions and other polymers, groupings at the oil/water interface allowing emulsion droplets to stabilise (Kinsella, 1990; Morr and Ha, 1993; Walstra, 1988), represent many of the characteristics that are desirable for a wall material. However, the literature on the use of food

proteins as coating materials for flavourings is rather scanty. Studies of interactions between various food proteins and volatile compounds have shown that proteins usually possess a high binding capacity for the flavour constituents (O'Neil, 1996). It was found that the flavour binding mechanism to proteins depends on the relationships between conformational states of proteins (Kinsella and Damodaran, 1980; 1981; O'Neil and Kinsella, 1987) and the nature of the aromatic compounds (Dufour and Haertle, 1990; Landy et al., 1995). Special attention has been given to β-lactoglobulin, the most abundant protein in whey. Papiz et al. (1986) postulated that it belongs to the superfamily of proteins involved in strong interactions with small hydrophobic molecules. Other studies have focused on the binding of selected flavour compounds, such as aldehydes, ketones, esters, acids, pyrazines, aromatic compounds or terpenes (O'Neil and Kinsella, 1987; Dufour and Haertle, 1990; Boudaud and Dumont, 1994; Charles et al., 1996; Jouenne and Crouzet, 1996; Pelletier et al., 1998). Hydrophobic interactions were found for ketones, esters and alcohols and covalent or hydrogen binding was found with aldehydes (Jasinski and Kilara, 1985; O'Neil and Kinsella, 1987; Dufour and Haertle, 1990; Charles et al., 1996; Pelletier et al., 1998). However, the data obtained often differed between these authors, due to different experimental conditions and methodologies being used. Some investigations have proved that milk proteins function well for encapsulating anhydrous milk fat, soybean, palm-based oils and methyl linoleate (Moreau and Rosenberg, 1993, 1996, 1998, 1999; Rosenberg and Young, 1993; Young et al., 1993a,b; Onwulata et al., 1994, 1996; Faldt and Bergenstahl, 1996a,b; Noor Lida Habi Mat Dian et al., 1996; Minemoto et al., 1997; McNamee et al., 1998; Sheu and Rosenberg, 1998; Pauletti and Amestoy, 1999). Nevertheless, on the basis of the chemical and physico-chemical properties of the investigated proteins, it is likely that they are suitable for being used as wall material for flavour encapsulation via spray drying. To our knowledge, flavour encapsulation into protein-based matrices has been comparatively little investigated. Also no reports have been found on the microencapsulation of many of the less commercially important essential oils (i.e. other than citrus, mint, onion and garlic). In 1996 the microencapsulation properties of gum arabic and several food proteins (whey protein isolate (WPI), soy protein isolate (SPI) and sodium caseinate (SC)) were investigated by Kim and Morr (1996) and Kim et al (1996). It was found that SPI was the most effective matrix for retaining orange oil during spray drying of the liquid orange oil emulsions (encapsulation effectiveness was 85.7%), followed by SC (81.7%), gum arabic (GA) (75.9%) and WPI (72.2%). Results of scanning electron

microscopy (SEM) and confocal scanning laser microscopy (CSLM) revealed that spray dried GA-microencapsulated orange oil particles had undergone more shrinkage during drying than the protein microencapsulated products.

This study investigates the encapsulating properties of milk proteins and some maltodextrins for the encapsulation of caraway (*Carum carvi* L.) essential oil, which is one of the most popular flavourings used in Lithuania. Evaluation of the effectiveness of the encapsulation process, resistance to oxidation, flavour profile and release by microencapsulated products, as well as the outer structure of the spray dried particles, were investigated.

MATERIALS AND METHODS

Materials

Whey protein concentrate (WPC) and skimmed milk powder (SMP) obtained from the local dairy; maltodextrins (MD): N-Lok (produced from waxy maize), Encaps-855 (produced from waxy maize) and Capsul-E (produced from tapioca) from National Starch & Chemical, USA; dried yeast autolysate (YA) and dried yeast extract (YE) from SEMA, Lithuania; corn starch (CS) and modified starch (MS) Ctex 06205 (an acetyled distarch adipate on waxy maize starch) from Cerestar; were used as encapsulating agents. Caraway essential oil was purchased from Frey+Lauhenstedtulzburg, Germany.

Emulsion preparation and drying

Solutions of the coating matrices, 30% concentration (w/w), were prepared by reconstituting dried powders of WPC and SMP in 50°C deionised water. Maltodextrins were added to protein matrices in the ratio 1:9 after being dissolved at 80°C. Solutions were allowed to cool to room temperature and were mixed overnight to enhance hydration. Caraway essential oil (15% w/w of matrix solids) was warmed to 40°C and emulsified into the hydrated coating material. Homogenisation was accomplished by Ultra Turrax Ika 25 basic homogeniser (Janke & Kunkel GmbH & Co. kg) operating at 20 000 rpm for 5 min. Emulsions were spray dried in

a Büchi 190 Mini Spray Dryer under the following conditions: spray nozzle (inlet) temperature $180\pm5°C$, outlet air temperature $90\pm5°C$; pressure 750–800 mm/H_2O. Dried products were packed into the glass containers and stored in laboratory freezer until evaluation.

Determination of total oil retained

The content of total oil retained after spray drying was determined by distilling 10g of encapsulated, dried powders for 3 h in a Clevenger-type apparatus (European Pharmacopoeia). The weight of oil recovered from the sample and collected in the trap was calculated by multiplying by a density factor of 0.88 g ml^{-1}. All samples were analysed in duplicate.

Determination of surface oil

Surface oil was washed for 4 h from 10 g samples of powder in a Soxhlet extraction apparatus using pentane. One millilitre of pentane containing 0.3% (v/v) decane as internal standard was added to the obtained extract prior to evaporation under nitrogen. Each extract was evaporated to a final volume of approximately 2 ml under a stream of nitrogen at room temperature. The amount of oil in the sample was determined by gas chromatography using a Fisons 8000 series chromatograph with FID under the following conditions: fused silica capillary column DB-5, 30 m length, 0.32 mm i.d. and 0.25 μm film thickness; helium as carrier gas with a linear velocity 35 cm s^{-1}; temperature programming from 50°C with 5 min hold to 220°C increasing at 4°C min^{-1}; injector temperature 230°C, detector 260°C.

Determination of moisture

Moisture was determined using the toluene distillation method. A 10 g sample of encapsulated caraway oil was refluxed with 100 ml toluene for 2.5 h in a boiling 250 ml flask fitted with a Bidwell-Sterling trap and a water-cooled condenser. The volume of the collected water was read directly from the trap.

Storage stability evaluation

Five g samples of each spray dried microencapsulated product were washed with pentane to remove the surface oil and were placed in separate 40 ml bottles, tightly capped and stored at 50°C in the dark, and at room temperature both in the presence and absence of light. For comparison, non-encapsulated caraway essential oil was stored in identical conditions.

Samples for gas chromatographic analysis were prepared according to the method of Risch and Reineccius (1988). A 0.15g sample of powder was dissolved in 0.85g distilled water. Then 4 ml acetone containing 0.3% (v/v) decane as internal standard was slowly added. Solutions were continuously shaken for 2 h, and then the sample was allowed to settle and 1 μl aliquot of the liquid phase was injected into the GC for limonene oxide content analysis without any further preparation. GC operating conditions were as described in the surface oil determination section.

Release of volatiles by dynamic headspace analysis (DHS)

The release of caraway oil volatiles from the microencapsulated product was determined by dynamic headspace analysis (DHS). Five g samples which had been extracted with pentane for the surface oil determination were placed into a 200 ml sample flask and flushed for 30–240 min with nitrogen carrier gas at a flow rate of 450 ml min^{-1} to recover the volatiles accumulated in the headspace during the timed interval. The volatiles trapped in 0.4g Tenax, TA 35/60 mesh. were desorbed with 15 ml of dimethyl ether. After adding 1 ml of internal standard (0.3% (v/v) 4-methyl-1-pentanol in dimethyl ether) to the desorbed volatiles, they were evaporated to a final volume of 1 ml under a stream of nitrogen and analysed by gas chromatography under the conditions described above.

Scanning electron microscopy (SEM)

A JEOL 840-A model scanning electron microscope was used to investigate the microstructural properties of spray dried microencapsulated products. Specimens were coated with gold with a

Balzers SCD 004 sputter coater. The operating conditions for the electron microscope were as follows: working distance 39 mm, acceleration voltage 10 kV.

RESULTS AND DISCUSSION

Retention of volatiles and encapsulation efficiency

Nine constituents were identified in caraway essential oil: α-pinene, sabinene, myrcene, limonene, *cis*- and *trans*-dihydrocarvone, carvone, dihydrocarveol and β-caryophyllene. It is well known that caraway essential oil consists mainly of limonene and carvone. The first compound is a hydrocarbon which is also the main constituent of citrus oils, the second is a terpene ketone, which is an important constituent of such aromatic plants as spearmint and dill. The main characteristics (total amount of retained oil, surface oil, effectiveness of encapsulation process and moisture) of the investigated spray dried encapsulated products are presented in Table 7.1. It should be noted that only traces of oil were hydrodistilled from corn starch (CS) and modified starch (MS) matrices after spray drying. Only a slight improvement was achieved by adding some maltodextrins (MD) to CS. Retention of oil in the MD was also low, up to 25% from the oil added to emulsion. The result shows that these matrices retained only small amounts of essential oil following the emulsification processes which were used here. Reineccius (1989) demonstrated that emulsion particle size has a significant effect on the retention of orange oil in some carbohydrate matrices: the coarser the emulsion (i.e. the larger the particle size), the poorer is the flavour retention. Since maltodextrins and corn starch have no good emulsification properties, they produce coarse emulsions and therefore poor flavour retention during drying. Fine homogenisation at elevated pressures was not applied to the preparation of emulsions in our study and this factor could be crucial to the losses of oil during spray drying. Because of their poor capacity to retain volatiles, the carbohydrate-based matrices were not considered for further investigation. Protein-based matrices were quite efficient in their retention of caraway essential oil during spray drying. WPC-based matrices retained more than 80% of the oil added to the emulsion. Among the two milk derivatives, SMP and WPC, the latter showed a higher retention capacity for microencapsulation of caraway essential oil. The retained amounts of volatiles in SMP and

WPC matrices were 76.12% and 80.71% (on the basis of the oil amount added to the emulsion), respectively.

Replacement of WPC by 10% of various maltodextrins led to an increase in retention of volatiles from 80.71% (WPC) to 87.85% (WPC+ MD N-Lok) during drying. Meanwhile the SMP matrices showed the opposite tendency: replacing SMP by all types of carbohydrates gave an inferior retention of volatiles, from 76.12% to 69.22% for SMP and SMP + Encaps-855, respectively. The amount of caraway oil retained on the surface of the capsules was not large and in most cases did not exceed 2.5% of the total oil, except when the YE matrix was used.

Table 7.1. Characteristics of caraway oil encapsulation with different matrices.

No	Encapsulating agent	Total oil (%)	Surface oil (%)	Encapsulation efficiency (%)	Moisture (%)
1.	Whey protein concentrate (WPC)	80.71	1.9	78.81	3.50
2.	WPC+MD N-Lok (9:1)	87.85	1.97	85.88	2.90
3.	WPC+MD Encaps-855 (9:1)	85.53	2.48	83.05	3.77
4.	WPC+MD Capsul–E (9:1)	84.11	2.36	81.75	2.73
5.	Skimmed milk powder (SMP)	76.12	1.98	74.14	3.34
6.	SMP+ MD N-Lok (9:1)	71.04	1.40	69.64	2.18
7.	SMP+ MD Capsul-E (9:1)	70.86	1.02	69.84	2.50
8.	SMP+ MD Encaps-855 (9:1)	69.22	1.16	68.06	3.02

Effectiveness of microencapsulation is the most important characteristic of the process, which can be calculated by subtracting surface oil from the amount of the total retained in the matrix oil or measured by hydrodistillation of essential oil from the matrix after washing out surface oil with an organic solvent. YA and all the WPC-based matrices (with and without MD) were the most effective matrices in our study (Table 7.1). Efficiency of the systems containing WPC with each of the carbohydrates (N-Lok, Encaps-855 and Capsul-E) was higher than that for WPC as a sole wall constituent, giving 85.88%, 83.05%, 81.75% and 78.81%, respectively. In general, effective characteristics of the proteins used in this study

corresponded to the total oil retained in the matrices, because the major part of the essential oil retained was entrapped in the capsules. Among other factors, the retention of the core material during microencapsulation by spray drying is affected by the properties and composition of the emulsion and by the drying conditions (Rosenberg et al., 1990). In light of the relatively high hydrophobicity of whey proteins, the addition of carbohydrates enhances the hydrophilic nature of the wall system, which might limit the accessibility of microencapsulated essential oil to the diffusion process during the distillation. In previously reported work (Young et al., 1993b), it was also demonstrated that the yield and efficiency of microencapsulation of anhydrous milk fat (AMF) might be enhanced by selection of wall components exhibiting different functional properties, e.g. carbohydrates combined with whey proteins. They demonstrated that micronencapsulation efficiency of systems containing whey protein isolate (WPI) and each of the surface-active carbohydrates NAT46 and ENC855 was higher (ca 87% and 89%, respectively) than those obtained for WPI or either of NAT 46 or ENC 855 carbohydrates as a sole wall material (respectively 35%, 31% and 35%).

However, the addition of MD to SMP did not increase the efficiency of the microencapsulation process. The efficiency values decreased from 76.12% for SMP to 71.04%, 70.86% and 70.86% for SMP+ N-Lok, SMP+Capsul-E and SMP+Encaps-855, respectively. Results suggest that in these systems adverse effects were introduced by the surface-active carbohydrates. The complexity of the properties of the encapsulating materials used and the processes involved produce rather complex results. Several theories have been developed to explain the retention of volatiles during the drying of food materials. Volatile losses during spray drying are associated not only with the interaction between the drying droplets and the hot air but also with the process of droplet formation-atomisation (Thijssen and Rulkens, 1968). In 1967, Menting and Hoogstad postulated that volatile materials can leave drying droplets until the termination of the first stage of drying process, i.e. until a crust forms around the droplets. Further losses can occur only if the volatiles can pass through the crust by means of diffusion in the solid or through pores or channels. On the basis of this explanation, the type of solids, their concentration and drying temperature are very important for their effect on crust formation. According to "selective diffusion theory", presented by Brooks (1965) and Rulkens and Thijssen (1967), the diffusion coefficients of water and volatiles are reduced as water concentration decreases due to drying. As a result of differences

in molecular weight of water and volatiles, the reduction in the diffusivity of the volatiles is more pronounced than that of water. Once the crust has formed, the diffusivity of the volatiles is so low that for all practical purposes the volatiles are entrapped in the drying solid matrix, while water can still diffuse through the crust. The crust therefore becomes effectively a selective membrane. Since all operating conditions of the drying process (rate and temperature) have been kept constant during the experiment, it might be that crust formation was playing a major role in obtaining different retention rates of encapsulated caraway oil. WPC, by possessing a higher content of carbohydrates, reduces the time for dry skin (crust) to form around the drying droplet (capsule); thus decreasing the loss of volatiles. Another assumption is that the superiority of WPC over the SMP material can be attributed to its relatively high lactose content. Higher amounts of lactose in WPC enhance the ratio of the capsule solidification during the drying process and essential oil droplets are locked in the dry matrix. Moreau and Rosenberg (1993) have studied the microstructure of whey protein/lactose-based spray dried microcapsules containing anhydrous milk fat (AMF). They found that partial replacement of WPI by amorphous lactose significantly limits the proportion of AMF that can be extracted from the capsules by an apolar solvent Because it is unlikely that only molecular diffusion controls retention, we assume that the loss of the volatiles in the SMP matrices might be the outcome of some other physico-chemical differences between SMP and WPC.

Release of volatiles by dynamic headspace analysis (DHS)

The results for recovery of limonene and carvone from microencapsulated and pentane washed products are shown in Fig. 7.1. It was determined as a function of nitrogen purge time. Results reveal that compounds were released at different rates by microencapsulated products. The range of released limonene and carvone was from 0.01 µg g^{-1} for WPC+MD N-Lok (9:1) to about 2.6 µg g^{-1} for SMP+MD Capsul-E (9:1) and from 0.12 µg g^{-1} for WPC+MD N-Lok to 3.54 µg g^{-1} for SMP+MD Encaps-855 (9:1), respectively. The addition of different types of maltodextrins to the WPC matrix significantly reduced the release of entrapped volatiles from capsules (from 1.15 to 0.016 µg g^{-1} and from 1.18 to 0.12 µg g^{-1} for limonene and carvone respectively) while the addition of maltodextrins to the SMP matrix in most cases resulted in a higher amount of released volatiles.
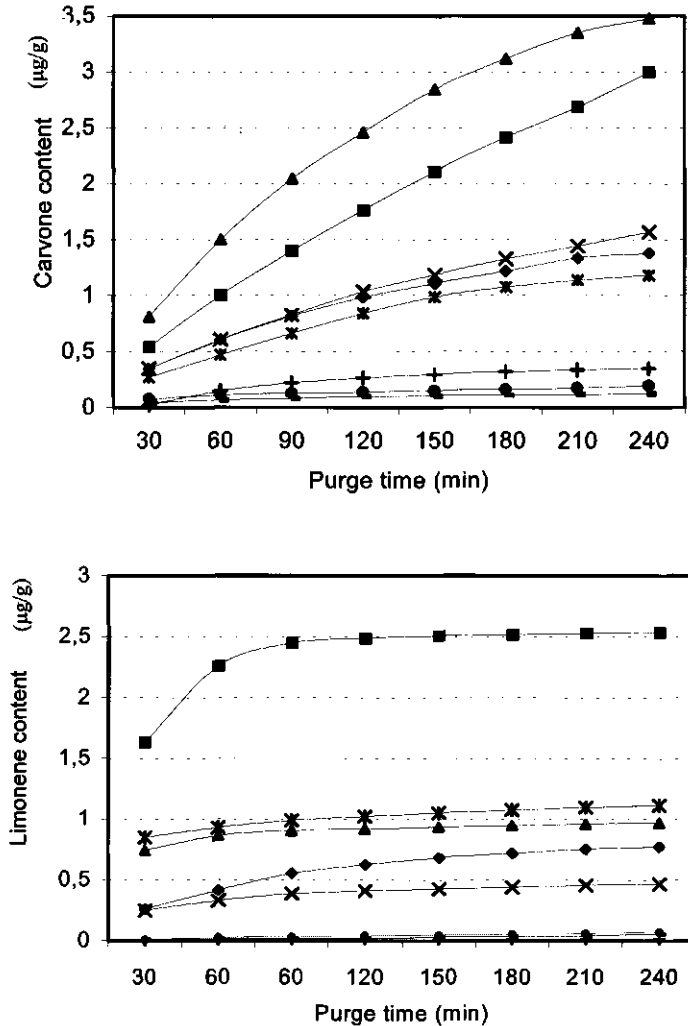
Fig. 7.1. Contents of limonene and carvone, released from microencapsulated caraway oil products by the DHS method: -♦- SMP; -■- SMP+MD Capsul-E; -▲- SMP+MD Encaps 855; ✱- SMP+MD N-lok; -✱- WPC; -●- WPC+MD Capsul-E; -+- WPC+MD Encaps 855; -−- WPC+MD N-lok.

The results also support the assumption that differences in the chemical composition of matrices influence the "locking" of entrapped droplets of essential oil in the capsule. It can be tentatively suggested that WPC applied together with MD can form a double layer around essential oil droplets, strongly protecting the release of its constituents during the purge of

nitrogen. Another assumption could be that lactose acts in its amorphous state as a hydrophilic filler or sealant that significantly limits diffusion of the volatiles through the walls of the capsule. However, further investigations with model systems consisting of separate fractions of WPC would be needed to prove or disprove this suggestion.

Flavour profile

The profile depends on the qualitative and quantitative composition of volatile constituents in the product. Therefore, it is important to measure the changes in these compounds during processing. Percentage concentrations of the principal constituents of caraway flavour (limonene and carvone), in pure non-encapsulated essential oil and hydrodistilled from the matrices after spray drying are shown in Fig. 7.2.
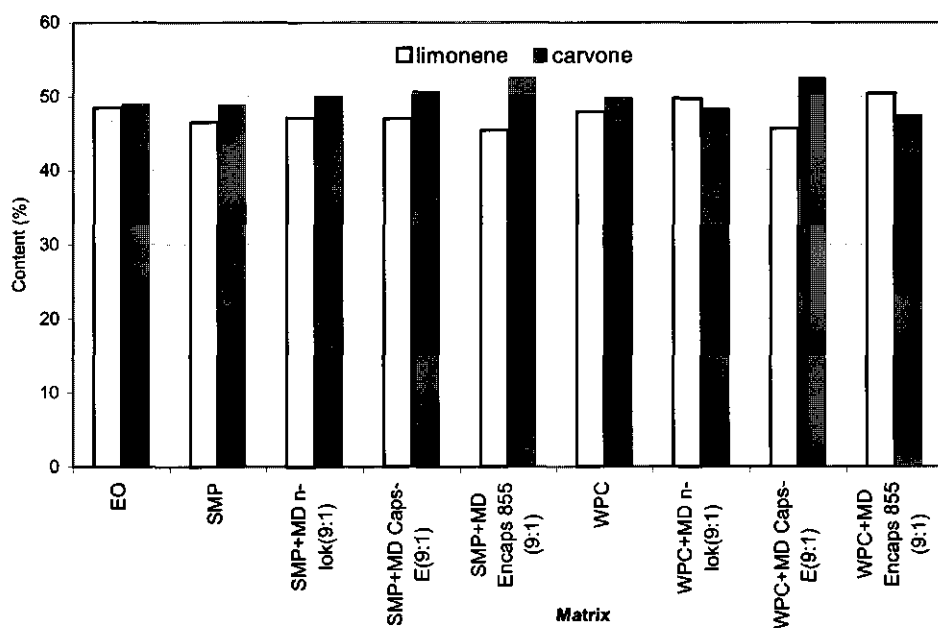
Fig. 7.2. Contents of limonene and carvone in pure caraway essential oil (EO) compared to retention in different matrices.

In general, the ratio of limonene to carvone in the matrices was similar to that in pure essential oil. A small decrease of limonene content in encapsulated oils has been recorded. This could

be a result of volatile losses during the drying process when emulsion droplets are in contact with high temperature air. Being more volatile, limonene is expected to be lost first. However, some increase in the percentage content of limonene was observed in two matrices.

Storage stability evaluation

The formation of oxidation compounds (limonene oxide isomers and tentatively identified as *cis*-para-menth-2-en-ol) was measured as a function of time for determining the protective properties of capsules during storage. Monitoring was carried out in different environmental conditions, i.e. 50°C and room temperature, and in the presence and absence of light. In parallel, samples of pure, non-encapsulated caraway essential oil were also exposed for the monitoring of formation of oxidation products.

Among eight different samples, SMP, SMP+N-Lok and WPC+N-Lok appeared to be least resistant to oxidation. The results of the storage stability test are shown in Figs 7.3–7.5. As expected, oxidation products were first recorded among the samples stored at 50°C. SMP and SMP+N-Lok encapsulated caraway essential oil products exhibited an induction period of 8 weeks during which no limonene oxide had been produced. By week 26, the value of *cis*-limonene oxide had reached levels of 2.13 and 1.89 mg g^{-1} oil for SMP and SMP+N-Lok samples, respectively. A similar content of *trans*-limonene oxide was produced by week 26 in SMP and SMP+N-Lok matrices – 2.22 and 2.34 mg g^{-1} oil. A WPC-based matrix with MD N-Lok contained *cis*- and *trans*-limonene oxides after 15 and 13 weeks, respectively. However, the amount of limonene oxides in WPC+N-Lok at the end of monitoring time was approximately four times less than in SMP and SMP+N-Lok, corresponding with 0.54 and 0.45 mg g^{-1} oil of *cis*- and *trans*-limonene oxide. Samples stored at room temperature showed longer times of resistance to oxidation. By week 20 of storage, 0.12 and 0.18 mg g^{-1} oil of *cis*-and *trans*-limonene oxides were recorded in SMP samples stored in the light. It seems that the oxidation process is influenced not only by temperature but also by the presence/absence of light. The same sample of SMP stored at room temperature without light exhibited a 3 week

Fig 7.3. cis- llmonene oxide
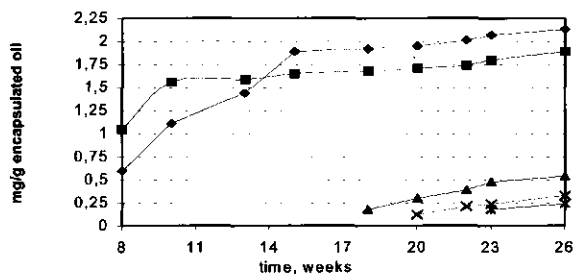
Fig. 7.4. trans-Limonene oxide
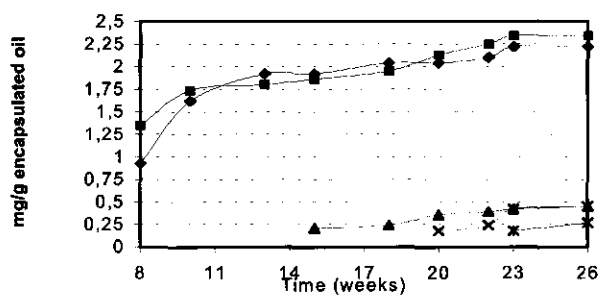
Fig. 7.5. cis-p-menth-2-en-ol
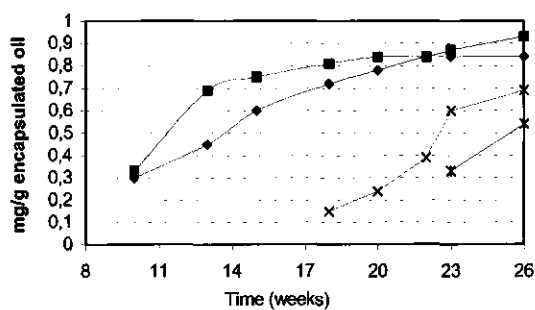
—◆— SMP stored at 50C

—■— SMP+N-lok stored at 50C

× SMP stored at room temperature with light

—✱— SMP stored at room temperature without light

—▲— WPC+N-lok stored at 50C

Figs 7.3–7.5. Formation of oxidation products (*cis*- and tr*ans*-limonene oxides, *cis*-p-menth-2-en-ol) in caraway essential oil encapsulated into different matrices during storage: 50°C, room temperature (RT) with/without light.

Fig. 7.6. cis-Limonene oxide
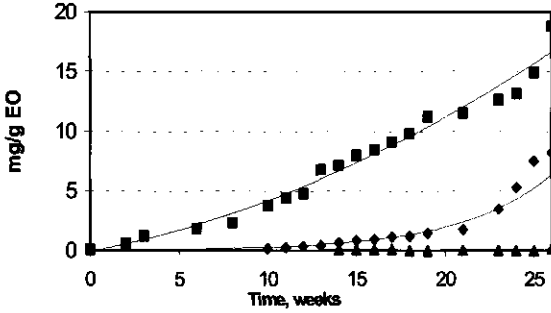
Fig. 7.7. trans-Limonene oxide
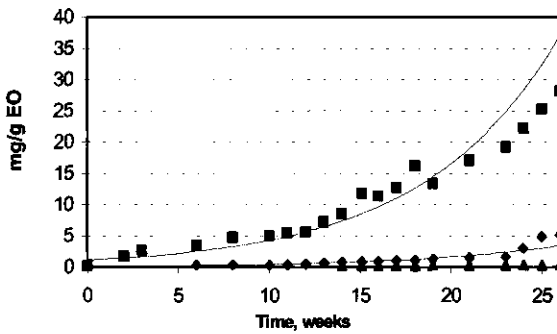
Fig. 7.8. cis-p-menth-2-en-ol
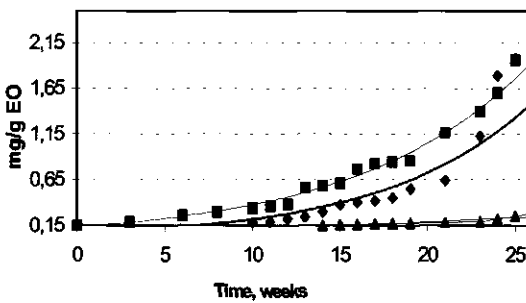
◆ t=50C ; ■ RT with light; ▲ RT without light;

Figs 7.6–7.8. Formation of oxidation products (*cis*- and *trans*-limonene oxides, *cis-p*-menth-2-en-ol) in non-encapsulated caraway essential oil during storage in different conditions: 50°C, room temperature (RT) with/without light.

longer induction period compared to that stored in the presence of light. However, the amounts of oxides recorded by week 26 were similar to those obtained at room temperature with light.

The third oxidation product was tentatively identified in our study as *cis*-p-menth-2-en-ol. It was found in SMP and SMP+N-Lok matrices at 50°C and in SMP samples stored at room temperature. The induction period of this component was 10 weeks for both samples of SMP and SMP+N-Lok stored at 50°C, and 18 and 23 weeks for SMP samples stored in the presence and absence of light. In general, all values of oxidation products formed in encapsulated oil were significantly lower than those in non-encapsulated oil.The finding that WPC-based microencapsulated products were consistently more stable against oxidation than SMP-based products is in agreement with the work of Kim and Morr (1996) in which WPI microencapsulated orange oil products were shown to be more stable against oxidation than sodium caseinate microencapsulated products.

Nevertheless, the results of storage of non-encapsulated caraway oil (Figs 7.6–7.8) in the same conditions as encapsulated oil, showed that the most significant influence of environment is light followed by temperature. The sample of caraway oil stored at room temperature in the presence of daylight exhibited a 3 week induction period for the formation of limonene oxides, reaching 18.82 and 28.22 mg g^{-1} oil values for *cis*- and *trans*-limonene oxides respectively by the end of monitoring. Meanwhile the oxidation of non-encapsulated oil was found to be significantly slower at 50°C: the induction period was 5 weeks and values of the oxides formed by the end of storage had reached 8.2 and 5.09 mg g^{-1} oil respectively, for *cis*- and *trans*-limonene oxide. From Figs 7.6–7.8 it is clear that the changes appearing in the oil stored at room temperature in the absence of light are not as significant as those occurring in the presence of light. This tendency is not clearly evident in encapsulated products when oil droplets are entrapped within the capsule and protected from the light by the wall. It implies that in this case photo-oxidation induced by light is more profound than oxidation by accelerated thermal degradation. This is important to bear in mind when handling and utilising caraway essential oil.
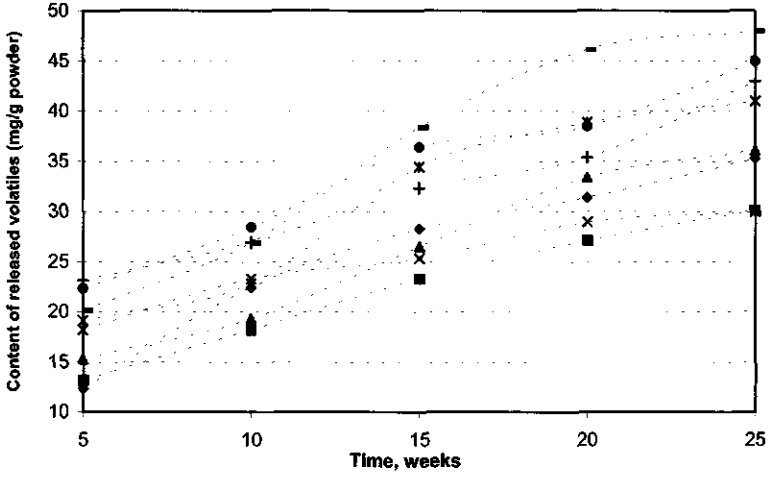
Fig. 7.9. Release of volatiles by different matrices during storage of powders at room temperature.
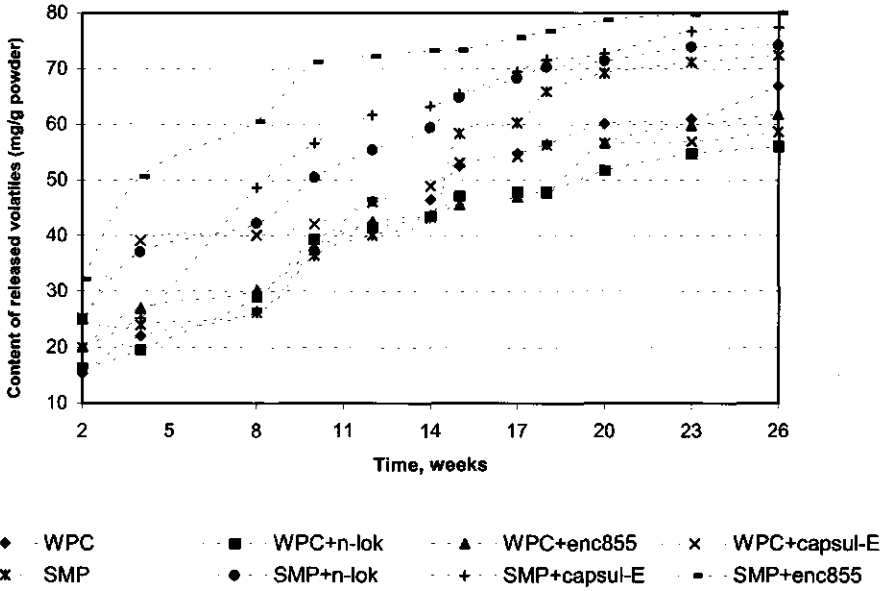

Fig. 7.10. Release of volatiles by different matrices during storage of powders at 50°C temperature.

Considering that there was no antioxidant in the encapsulated products, the storage stability is quite good for most of the products. Generally, the rate of formation of limonene oxide and other oxidation products may have been influenced by many factors such as matrix porosity to oxygen, absolute density, pro-oxidants, trace minerals or other compounds present. The role of entrained air (i.e. air included or trapped within the particle) in determining the storage stability of spray dried products has not been studied.

Release of volatiles during storage

Figures 7.9–7.10 represent the dynamics of the release of the aromatic volatiles from encapsulated powders as a function of time. A comparison was made between powders stored at 50°C and room temperature. As expected, lower losses of volatiles were found in the powders stored at room temperature than at 50° C. However, qualitative results of aroma losses during storage follow the same tendency as the analysis of volatile release by DHS and as monitoring of storage stability: SMP-based matrices exhibit higher volatile release and losses during storage time than WPC.

Microstructure

The outer topography of spray dried microcapsules was revealed by electron microscopy and is presented in Figs 7.11–7.16. In order to study the effect of the wall composition on the microstructural features of the spray dried microcapsules containing caraway oil, the same atomisation and drying conditions were consistently maintained in this study. SEM results for spray dried WPC-based matrices (Figs 7.11–7.12) revealed spherical particles with smooth surfaces and large variations in size. However, the surface of a few WPC capsules (Fig. 7.11) exhibited some holes. The matrices of WPC combined with carbohydrates (Fig. 7.12) did not differ greatly from sole WPC matrices, they contained fewer visible cracks although more dented surfaces could be observed in them compared to sole WPC. Wall composition, atomisation and drying parameters, and uneven shrinkage at the early stages of drying were attributed to factors affecting the formation of surface indentations in spray dried particles (Buma and Henstra, 1972; Rosenberg et al., 1988; Mistry et al., 1992; Rosenberg and Young, 1993). In 1998, Sheu and Rosenberg studied how the structure of spray dried microcapsules

with wall materials consisting of whey proteins and carbohydrates were affected by WPI:COH ratio and by the carbohydrate profile. Results of this work suggested that the extent of surface indentation was inversely related to the content of WPI included in the wall. Another observation made in this study was the effect of the ratio of high-to-low molecular weight (MW) solutes (included in the wall) on the structure of microcapsules: as DE value increased, the proportion of capsules with caps also increased. This tendency to develop surface dents affected by the ratio of low-to-high molecular weight solutes that form viscoelastic properties of drying solution or emulsion as noticed by Rosenberg and Young (1993). Although the capsules were prepared from emulsions that differed in their composition by means of different added maltodextrins, no significant differences in outer topography could be detected.

The surfaces of capsules with SMP-based walls were different from WPC; the surface of capsules with sole SMP (Fig. 7.13) had fewer surface dents, cracks and wrinkles compared to SMP combined with carbohydrates (Fig. 7.14). The presence of surface dents reported for spray dried skimmed milk powders has been attributed to the effect of drying on casein, conditions of atomisation and drying (Buma and Henstra, 1971). They suggested that the observed surface folds, pores and cracks represented the effects of mechanical stresses induced by uneven drying at different parts of the drying droplets by shrinkage of casein. They also concluded that casein rather than lactose was probably responsible for surface dents.

Figures 7.15–7.16 represent the inner structure of shattered SMP and WPC capsules and show the porous structure of the interior regions of the spray dried particle wall. Caraway oil (O) is organised in the form of small droplets embedded in the capsule wall (W). The existence of holes (H) on the central void (CV) surface is evident. The nature of these features is not completely clear, since there is conflicting information regarding the role of different milk constituents in affecting particle structure.
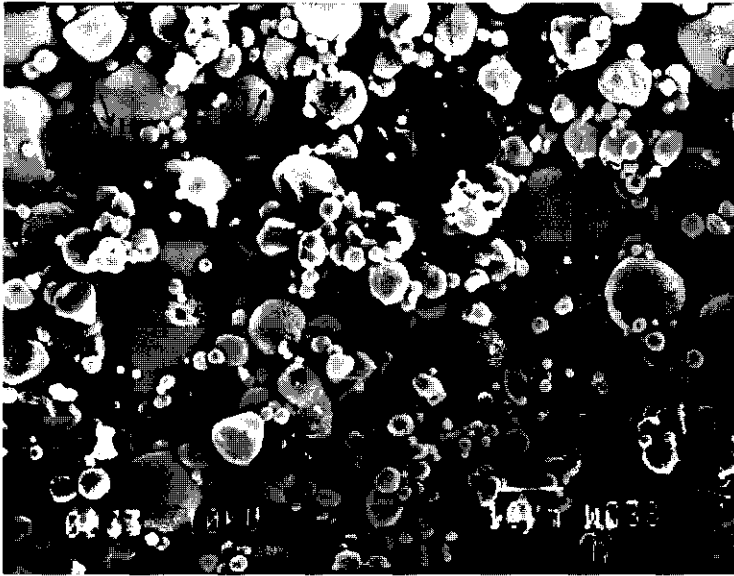
Fig. 7.11 Micrographs of spray dried WPC based caraway oil containing microcapsules. H=hole. Scale bar=10μm.
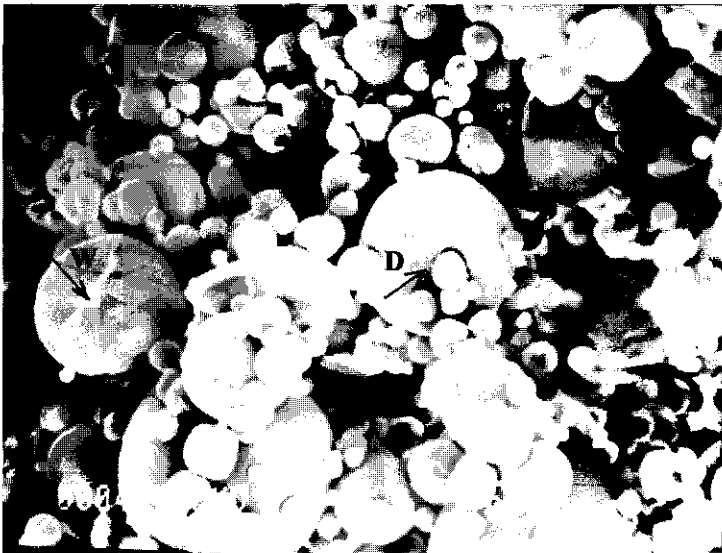
Fig. 7.12. Micrographs of spray dried WPC + MD Capsul-E--based caraway oil containing microcapsules. W = wrinkle, D = dent. Scale bar = 10 μm.
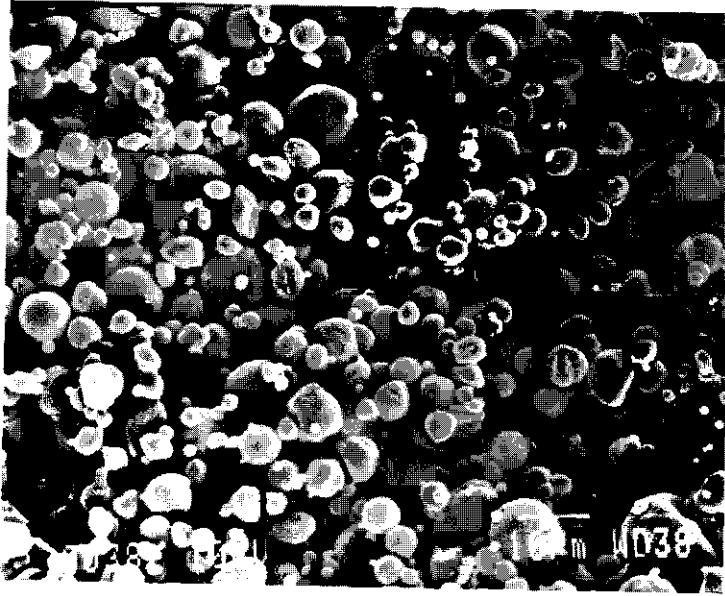
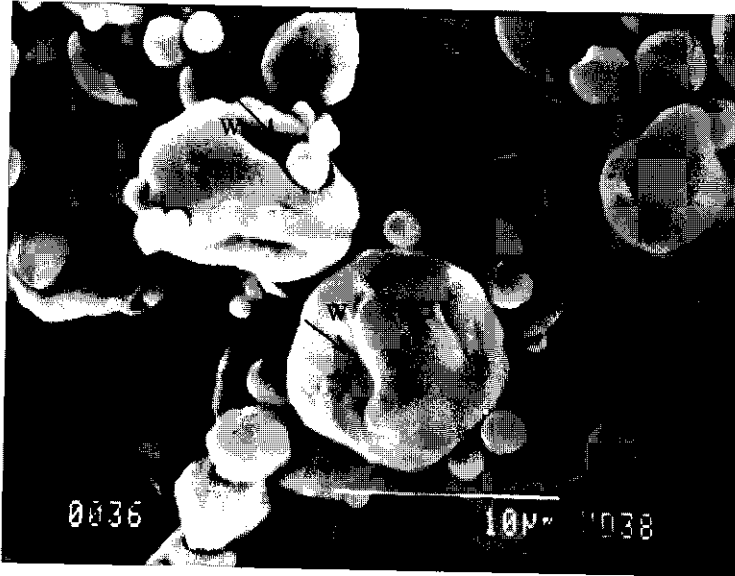**Fig. 7.13.** Micrographs of spray dried SMP based caraway oil containing microcapsules. Scale bar=10μm.

Fig. 7.14. Micrographs of spray dried SMP+MD Encaps 855 based caraway oil containing microcapsules. W=wrinkle. Scale bar=10μm.

Fig. 7.15. Micrograph revealing the inner structure of microencapsulated to WPC caraway oil matrix: W=wall of the matrix, H=hole, O=oil droplets. Scale bar=10μm.
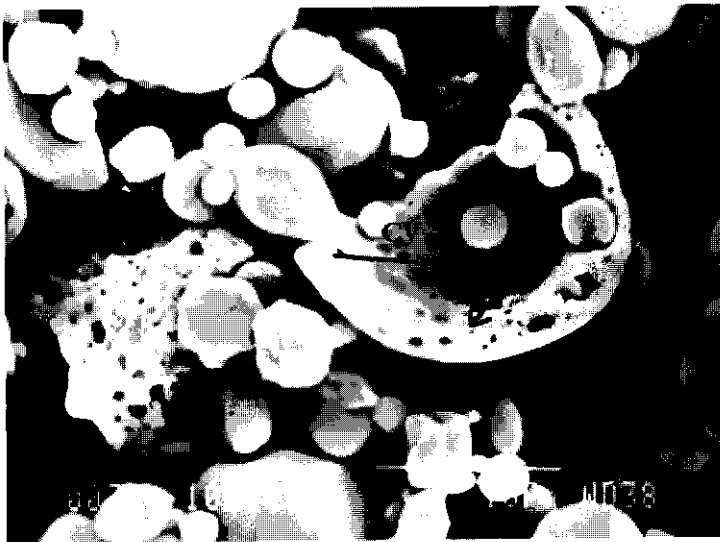
Fig. 7.16. Micrograph revealing the inner structure of microencapsulated to SMP caraway oil matrix: CV=central void, IS=inner surface. Scale bar=10μm.

Damodaran, S.; Kinsella, J.E. Flavor protein interactions. Binding of carbonyls to bovine serum albumin: thermodynamic and conformational effects. *J.Agric. Food Chem.* **1980**, 28, 567-571.

Damodaran, S.; Kinsella, J.E. Interaction of carbonyls with soy protein: conformational effects. *J.Agric. Food Chem.* **1981**, 29, 1253-1257.

Dziezak J.D. Microencapsulation and encapsulated ingredients. *Food technol* **1988**, 42, 136-151.

Dufour, E.; Haertle, T. Binding affinities of beta-ionone and related flavor compounds to β-lactoglobulin:effects of chemical modifications. *J.Agric. Food Chem* **1990**, 38, 1691-1695.

Charles, M.; Bernal, B.; Guichard, E.; Interactions of β-oactoglobulin with flavour compounds. In Developments in Flavour Science; Taylor, A.J., Mottram, D.S., Eds.; The Royal Society of Chemistry: London, U.K., 1996; pp 433-436.

Faldt, P.; Bergenstahl, B. Spray-dried whey protein/lactose/soybean oil emulsions.1. Surface composition and particle structure. *Food Hydrocolloids* **1996a**, 10,421-429.

Faldt, P.; Bergenstahl, B. Spray-dried whey protein/lactose/soybean oil emulsions.2. Redispersibility, wettability and particle structure. *Food Hydrocolloids* **1996b**, 10,431-439.

Faldt, P.; Bergenstahl, B. The surface coverage of fat on food powders analyzed by ESCA (Electron spectroscopy for chemical analysis). *Food structure*, **1993**, 12, 225-234.

Franzen, K.L; Kinsella, J.E. Parameters affecting the binding of volatile flavour compounds in model food systems. I. Proteins. *J. Agric. Food Chem.* **1974**, 22, 675-678.

Jackson, L.S.; Lee, K. Microencapsulation in food industry. *Lebensm.-Wiss. u.-Technol.* **1991**, 24, 289-297.

Jasinski, E.; Kilara, A. Flavour binding by whey proteins. *Milchwissenschaft* **1985**, 40, 596-599.

Kim Y.D.; Morr C.V; Schenz T.W. Microencapsulation properties of gum arabic and several food proteins: liquid orange oil emulsion particles. *J.Agric.Food Chem.* **1996**, 44,1308-1313.

Kim, Y.D.; Morr, C.V. Microencapsulation properties of gum arabic and several food proteins: spray-dried orange oil emulsion particles. *J. Agric. Food Chem.* **1996,** 44, 1314-1320.

Kollengode, A.N.R.; Hanna, M.A. Flavor retention in pregelatinized and internally flavored starch extrudates. *Cereal Chem.* **1997,** 74, 396-399.

Kinsella, J.E. Milk proteins: physicochemical and functional properties.*Crit. Rev. Food Sci. Nutr.* **1990,** 21,197-262

Landy P., Druaux C., Voilley A. Retention of aroma compounds by proteins in aqueous solution. *Food Chem.* **1995,** 54, 387-392.

McNamee, B.F.; O'Riordan, E.D.; O'Sullivan, M. Emulsification and microencapsulation properties of gum arabic. *J. Agric. Food Chem.* **1998,** 46, 4551-4555.

Menting, L.C.; Hoogstad, B. Volatile retention during the drying of aqueous carbohydrate solutions. *J.Food Sci.* **1967,** 32, 87-90.

Minemoto, Y.; Adachi, S.; Matsuno, R. Comparison of oxidation of methyl linoleate encapsulated with gum arabic by hot-air-drying and freeze-drying. *J. Agric. Food Chem.* **1997,** 45, 4530-4534.

Mistry, V.V.; Hassan, H.N.; Robinson, D.J. Effect of lactose and protein on microstructure of dried milk. *Food Struct.* **1992,** 11, 73-82.

Moreau, D.L.; Rosenberg, M. Oxidative stability of anhydrous milkfat microencapsulated in whey proteins. *J. Food Sci.* **1996,** 61, 39-43.

Moreau, D.L.; Rosenberg, M. Microstructure and fat extractability in microcapsules based on whey proteins or mixtures of whey proteins and lactose. *Food structure,* **1993,** 12, 457-468.

Moreu, D.L.; Rosenberg, M. Porosity of whey protein-based microcapsules containing anhydrous milkfat measured by gas displacement pycnometry. *J. Food Sci.* **1998,** 63, 819-823.

Moreu, D.L.; Rosenberg, M. Porosity of microcapsules with wall systems consisting of whey proteins and lactose measured by gas displacement pycnometry. *J. Food Sci.* **1999,** 64, 405-409.

Noor Lida Habi Mat Dian, Nor'aini Sudin; Mohd Suria Affandi Yusoff. Characteristics of microencapsulated palm-based oil as affected by type of wall material. *J.Sci Food Agric*, **1996**, 70, 422-426.

O'Neil, T.E. Flavour binding by food proteins: an overview. In *Flavor Food Interaction*; McGorrin, R.J., Leland, J.V., Eds., ACS Symposium Series 633; American Chemical Society: Washington, DC, 1996; pp59-74.

O'Neil, T.E.; Kinsella J.E. Binding of alkanone flavors to β-lactoglobulin: effects of conformational and chemical modification. *J.Agric. Food Chem.* **1987**, 35, 770-774.

Onwulata, C.I.; Smith, P.W.; Craig, Jr.J.C.; Holsinger, V.H. Physical properties of encapsulated spray-dried milkfat. *J. Food Sci.* **1994**, 59, 316-320.

Onwulata, C.I.; Smith, P.W.; Cooke, P.H.; Holsinger V.H. Particle structures of encapsulated milkfat powders. *Lebensm.- Wiss. u. - Technol.*, **1996**, 29, 163-172.

Papiz, M.Z.; Sawyer, L.; Eliopoulos, E.E.; North, A.C.T.; Findlay, J.B.C.; Sivaprasadarao, R.; Jones, T.A.; Newcomer, M.E.; Kraulis, P.J. The structure of β-lactoglobulin and its similarities to plasma retinol binding protein. *Nature* **1986**, 324, 383-385.

Pauletti, M.S.; Amestoy, P. Butter microencapsulation as affected by composition of wall and fat. *J. Food Sci.* **1999**, 64, 279-282.

Pelletier, E.; Sostmann, K.; Guichard, E. Measurement of interactions between β-lactoglobulin and flavor compounds (esters, acids and pyrazines) by affinity and exclusion size chromatography. *J.Agric.Food Chem.* **1998**,46, 1506-1509.

Reineccius, G.A. Carbohydrates for flavor encapsulation. *Food Technol.* **1991**, March, 144-149.

Reineccius, G. A. Flavor encapsulation. *Food Reviews International* **1989**, 5, 147-176.

Reineccius, G.A. Spray drying of food flavors. In *Flavor encapsulation*, eds S.J. Rish, G.A. Reineccius. ACS symposium series 370; American Chemical Society: Washington, D.C., 1988; pp 57-66.

Rish, S.J.; Reineccius, G.A. Spray-dried orange oil: effect of emulsion size on flavor retention and shelf stability. In *Flavor encapsulation*, eds S.J. Rish, G.A. Reineccius. ACS symposium series 370; American Chemical Society: Washington, D.C., 1988; pp 67-77.

Rish, S.J. Encapsulation: overview of uses and techniques. In *Encapsulation and controlled release of food ingredients*, eds S.J. Rish, G.A. Reineccius. ACS symposium series 590; American Chemical Society: Washington, D.C., 1995; pp 2-7.

Rosenberg, M.; Kopelman, I.J.; Talmon, Y. Factors affecting in spray-drying microencapsulation of volatile materials. *J.Agric.Food Chem.* **1990**, 38, 1288-1294.

Rosenberg, M.; Talmon, Y.; Kopelman, I.J. The microstructure of spray- dried microcapsules. *Food Microstruc.* **1988**, 7, 15-23.

Rosenberg, M.; Young, S.L. Whey proteins as microencapsulating agents. Microencapsulation of anhydrous milkfat - structure evaluation. *Food Structure* **1993**, 12, 31-41.

Rulkens, W.H.; Thijssen, H.A.C.; The retention of organic volatiles in spray drying aqueous carbohydrate solutions. *J.Food Technol.* **1967**, 7, 95-105.

Sheu, T.Y.; Rosenberg, M. Microencapsulation of volatiles by whey proteins. *J. Dairy Sci.* **1993**, 76 (Suppl. 1), 106.

Sheu, T.Y.; Rosenberg, M. Microstructure of microcapsules consisting of whey proteins and carbohydrates. *J. Food Sci.* **1998**, 63, 491-494.

Thenevet, F.Acacia gums. Natural encapsulation agent for food ingredients. In *Encapsulation and controlled release of food ingredients*, eds S.J. Rish, G.A. Reineccius. ACS symposium series 590; American Chemical Society: Washington, D.C., 1995, pp 51-59.

Thijssen, H.A.C.; Rulkens, W.H. Retention of aromas in drying food liquids. *Ingenieur* **1968**, 80, Chapter 45.

Trubiano, P.C.; Lacourse, N.L. Emulsion-stabilizing starches. Use in flavor encapsulation. In *Flavor encapsulation*, eds S.J. Rish, G.A. Reineccius. ACS symposium series 370; American Chemical Society: Washington, D.C., 1988; pp 45-54.

Young, S.L.; Sarda, X.; Rosenberg, M. Microecpasulating properties of whey proteins. 1.Microencapsulation of anhydrous milk fat. *J.Dairy Sci.* **1993a**, 76, 2868-2877.

Young, S.L.; Sarda, X.; Rosenberg, M. Microencapsulating properties of whey proteins. 2. Combination of whey proteins with carbohydrates. *J.Dairy Sci.* **1993b**, 76, 2878-2885.

Zeller, B.L.; Saleeb, F.Z.; Ludescher R.D. Trends in development of porous carbohydrate food ingredients for use in flavor encapsulation. *Trends Food Sci. Technol.* **1999**, 9, 389-394

GENERAL DISCUSSION

This thesis deals with research into aroma with regard to some plants of the *Umbelliferae* and *Asteraceae* families. The following investigations were included within the framework of the experimental design:

- A study of aroma composition in plants using instrumental analysis. The following factors influencing the aroma composition of essential oils from particular plants were examined: cultivar, fertilisation rate, harvesting time, and anatomical part of the plant used for the isolation of volatiles (Chapters 2, 3, 4 and 5).
- Flavour release from dried plant and microencapsulated products with respect to harvesting time, anatomical part of plant, the effect of different matrices being used for encapsulation, and the detection of odour-active components by sniffing port analysis (Chapters 5 and 7).
- The processing of essential oils by emulsification and microencapsulation (Chapters 6 and 7).

AROMA COMPOSITION OF PLANTS

In this study, the composition of essential oils from aromatic plants grown in Lithuania was shown to be influenced by cultivar, fertilisation rate, plant harvesting time and the anatomical part of the plant exploited for the isolation of volatile compounds.

Essential oils are the odorous principles found in various plant parts as compounds of secondary plant metabolism. Depending on the plant family, volatile oils are secreted in oil cells, in secretion ducts or in cavities or glandular hairs (Trease and Evans, 1983).

Compositional variation within a species appears to be the rule rather than the exception in essential oil crops (Franz, 1993). It involves the study of at least three factors: (1) individual genetic variability; (2) variation among different plant parts and different developmental stages; and (3) modifications due to the environment.

Plants grown at different locations and in different climatic conditions differ in their aroma composition (Halva et al., 1988; Galambosi and Peura, 1996; Perry et al., 1999). Accumulation of essential oil increases with light and temperature (Haelvae et al., 1992, 1993). There is an established positive relationship between the intensity of solar radiation

and the quantity of phenolic formation by the plant (Waterman and Mole, 1994). However, the concentration of diterpenes in plants decreases in response to light and water stress (Lewinsohn et al., 1993; McGarvey and Croteau, 1995). Using nitrogen fertilisers during plant cultivation can increase the yield of essential oils (Hussien, 1995; Muni Ram et al., 1997).

Different yields of the essential oils with variations in their composition can be obtained from aromatic plants by harvesting them during their vegetative period (Senatore, 1996; Chalcat et al., 1997; Mallavarapu et al., 1999; Sefidkon et al., 1999). The trends for the accumulation of essential oil with optimal yield and composition in the glands of the plant can differ between species, so each particular crop requires investigation in order to obtain the desired aromatic compounds (Faber et al., 1997; Perry et al., 1999; Munne-Bosch, 2000).

The yield and composition of volatiles can vary significantly according to which botanical part of the plant is selected (Fiorini et al., 1997). Depending on the family, volatiles may occur in secretory structures such as glandular hairs (*Labiatae*), oil-tubes called vittae (*Umbelliferae*) or lysigenous passages (*Pinaceaae*) (Tyler et al., 1981). Some plants possess secretory ducts only in particular organs and this is why the formation of volatile oils differs according to which part of the plant is used.

In the present work the qualitative composition of essential oils obtained from plants grown in the Lithuanian climate was shown to be similar to those grown in other locations except for some minor components (Chapters 2, 3 and 4). However, the differences in quantitative composition of volatiles were more evident. The time of harvesting appeared to be most significant factor for the yield of essential oil accumulated in the plant (Chapters 3 and 4) or the amount of volatiles released by plant material in certain conditions (Chapter 5). Differences in the yields of essential oil were also influenced by cultivar and fertilisation rate (Chapter 2).

Seasonal changes were found to have an important influence on quantitative composition, whereas qualitative characteristics remained the same during the whole vegetative period for the plants analysed (Chapters 3 and 4). A similar tendency of plants to accumulate highest amount of volatiles at the stage of flower-head emergence or at full flowering was observed

by Senatore (1996) and Mallavarapu et al. (1999) for thyme and davane essential oils, respectively. In general it is accepted that the most herbs accumulate the highest amounts of volatile oil during the anthesis period; however, numerous studies show that each particular aromatic plant possesses its own peculiarities.

Different yields and composition of volatiles were obtained from different botanical parts of the plants (Chapters 3, 4 and 5). As expected, the highest amounts of essential oils were accumulated by seeds and flowers, followed by leaves and stems. Remarkable differences appeared between representatives of the *Umbelliferae* and *Asteraceae*. Umbellifers were found to possess significant amounts of volatiles in all above-ground anatomical parts of the plant (Chapters 3 and 5), whereas only traces of volatiles were discovered in the stems of *Asteraceae* plants (Chapter 4).

FLAVOUR RELEASE

One of the methods most often applied in order to recover volatiles from plant material is distillation, which generally gives the finest quality oils and complete separation of non-volatile components (Heath and Reineccius, 1986). However, steam- or water-distilled essential oils do not necessarily reflect the composition of the actual odour of an aromatic plant. Essential oils are mostly artefacts formed during the distillation process (Baser, 1995). The identification of volatile compounds which are perceived by the human nose in the air above the food is a goal of flavour analysis (Guth and Grosch, 1993). The headspace techniques aim to capture the real odoriferous characteristics of the product. Static headspace provides an accurate composition of the volatile compounds which are perceived as the wholesome odour of the product, but it is limited to the level of detection and identification of organic constituents, particularly less volatile substances (Arino et al., 1999). Dynamic headspace (DHS) techniques permit the collection of larger amounts of volatile components for their qualitative and quantitative characterisation by instrumental methods. Besides the widely used instrumental detectors, the human olfactory system can be successfully used to obtain important information about the qualitative contribution of individual compounds to the overall flavour of the product (Acree and Barnard, 1994).

However, the release of volatiles depends not only on the method of isolation but on the structure and properties of the matrix in which the aroma compounds are presented: availability of the odorants to the vapour phase, and therefore on their affinity for the product (Druaux and Voilley, 1997).

In the present work, dynamic headspace–gas chromatography (DHS–GC) and dynamic headspace–gas chromatography–sniffing port (DHS–GC–SP) techniques were applied for the characterisation of flavour release from dried plant material and flavour-encapsulated matrices. The results revealed that the release of aroma compounds was influenced by the structure and composition of the plant material or the matrices of microencapsulated products (Chapters 5 and 7). The structure of the anatomical parts of plant had a significant influence on the amount and profile of released volatiles (Chapter 5). Roots exhibited the lowest amounts of released volatiles, whilst seeds appeared to possess the highest. In general, seasonal changes in the amount of volatiles determined by DHS (Chapter 5) are in a good agreement with the changes in the content of essential oils (Chapter 3). As expected by comparing composition of essential oils with those of DHS samples, the latter were dominated by compounds of higher volatility. The limited capture and detection of less volatile compounds by the DHS method was evident in this work (Chapter 5). Characterisation of effluents by GC–SP gave additional information on the components contributing to the flavour of lovage plants (Chapter 5).

Different microencapsulated products exhibited different rates of release of aroma compounds from the protein-based matrices (Chapter 7). Results imply that the mechanism of flavour release is a sum of factors. However, by making a proper selection of encapsulating matrix, flavour release and losses under certain conditions or storage can be controlled and reduced (Chapter 7).

FLAVOUR PROCESSING

Besides the advantages of being natural and possessing a flavour quality corresponding with the source raw material, the essential oils are volatile and labile compounds that are difficult to handle in their liquid, concentrated state and which are prone to oxidation. In order to

minimise the danger of this happening, to retain and protect volatile compounds and facilitate easier handling, flavours are converted to free-flowing powders by emulsification and encapsulation for use in numerous formulated products on the market.

In the present work, investigations into the properties of essential oils in water emulsions stabilised by protein and lipid showed that the stability of essential oil emulsion is lower than those obtained with emulsions of a purified olive oil as dispersed phase (Chapter 6). The stability of emulsions with essential oil can be promoted by increasing protein concentration and adding emulsifier to provide a decrease in particle size and a lower rate of creaming. The advantages of creating smaller emulsion droplets when preparing solutions for spray drying were reported by Rish and Reineccius (1988). Creation of a fine emulsion increased the retention of essential oil in the spray-dried powders which then yielded less extractable surface oil. Our investigation of the properties of microencapsulated products showed that by making a careful choice of carrier or combining wall materials with different functional properties, the efficiency of the microencapsulation process can be improved and subsequent flavour loss during storage can be minimised (Chapter 7). This is in agreement with Young et al. (1993) who also demonstrated that the yield and efficiency of microencapsulation of anhydrous milk fat might be enhanced by selection of wall components exhibiting different functional properties. Other factors such as environmental conditions also influence the retention of flavour during storage and the formation of oxidation products.

However, it is important to note that a flavour profile can undergo certain changes during its processing. A small decrease in the more volatile components has been recorded in encapsulated oils, which could be the result of volatile losses during the drying process.

Finally the study of the oxidation process occurring in essential oils during storage revealed that the formation of oxidation products is significantly slower in microencapsulated than in non-encapsulated oils.

CONCLUSIONS

The following conclusions can be drawn from the thesis "*Aroma of some plants cultivated in Lithuania: composition, processing and release*":

- In general, the chemical composition of locally grown aromatic plants (parsley, celery, dill, caraway) shows that the amounts of the major constituents were arranged in the same order as that reported in the literature. The yield and chemical composition of the essential oils (caraway, lovage, costmary) are influenced by factors such as type of cultivar, cultivation site, rate of fertilisation, anatomical part of plant used, and harvesting time. Essential oils produced from locally grown plants can successfully replace imported oils.

- Different detection techniques for the effluents of a GC column, for instance the DHS–GC–SP technique, can provide additional information about the contribution of the individual volatiles to the flavour of a plant. The method used to isolate the volatiles has a significant influence on their composition: 98 compounds were identified in the samples, 41 of which are reported among lovage volatiles for the first time.

- Emulsions made with triglyceride oil are more stable than those made with caraway essential oil as the dispersed phase. The stability of the essential oil-in-water emulsions can be improved considerably by adding soyabean phosphatidylcholine (sb-PC) and increasing the protein concentration. Adsorption of the lipid from the oil and the protein from the aqueous phase at the oil/water interface can be studied by applying ellipsometry.

- The essential oil of caraway can successfully be encapsulated in milk derivatives either alone or in combination with carbohydrates. The yield and efficiency of microencapsulation of essential oils could be enhanced by the selection of wall components exhibiting different functional properties.

REFERENCES

Acree, T. E.; Barnard, J. Gas chromatography-olfactometry and Charm Analysis. In *Trends in Flavour Research*; Maarse, H., Van der Heij, D. G., Eds.; Elsevier Science Publishers B. V.: Amsterdam, 1994; pp 211-220.

Arino, A.; Arberas, I.; Renobales, G.; Dominguez, J.B. Influence of extraction method and storage conditions on the volatile oil of wormwood (*Artemisia absinthium* L.). *Eur. Food Res. Technol.* **1999**, 209, 126–129.

Baser, H.C.K. Analysis and quality assessment of essential oils. In *A manual on the essential oil industry,* Tuley de Silva (ed.); United Nations industrial development organization, Vienna, 1995; pp 155-178.

Chalcat, J.C.; Michet, A.; Pasquier, B. Influence of harvesting time on chemical composition of *Mentha piperita* L. essential oil. *Perfum. Flavor.* **1997**, 22, 17–21.

Druaux, C.; Voilley, A. Effect of food composition and microstructure on volatile flavour release. *Trends Food Sci. Technol.* **1997**, 8, 364–368.

Faber, B.; Bangert, K.; Mosandl, A. GC-IRMS and enantioselective analysis in biochemical studies in dill (*Anethum graveolens* L.). *Flavour Fragrance J.* **1997**, 12, 305–314.

Fiorini, C.; Fouraste, I.; David, B.; Bessiere, J.M. Composition of the flower, leaf and stem essential oils from *Laurus nobilis* L. *Flavour Fragrance J.* **1997**, 12, 91–93.

Franz, Ch. Genetics. In *Volatile oil crops: their biology, biochemistry and production*; Hay, R.K.M., Waterman, P.G.; Eds.; Longman: Harlow, U.K., **1993**; pp 63–96.

Galambosi, B.; Peura, P. Agrobotanical features and oil content of wild and cultivated forms of caraway (*Carum carvi* L.). *J. Essent. Oil Res.* **1996**, 8, 389–397.

Guth, H.; Grosch, W. Identification of potent odourants in static headspace samples of green and black tea powders on the basis of aroma extract dilution analysis (AEDA). *Flavour Fragrance J.* **1993**, 8, 173–178.

Halva, S.; Huopalahti, R.; Franz, R.; Makinen, S. Herb yield and essential oil of dill (*Anethum graveolens* L.) at different locations. *J. Agric. Sci. Finland* **1988**, 60, 93–100.

Haelvae, S.; Craker, L.E.; Simon, J.E.; Charles, D.J. Light levels, growth and essential oil in dill (*Anethum graveolens* L.). *J. Herbs Spices & Med. Plants* **1992**, 1, 47–58.

Haelvae, S.; Craker, L.E.; Simon, J.E.; Charles, D.J. Growth and essential oil in dill, *Anethum graveolens* L., in response to temperature and photoperiod. *J. Herbs Spices & Med. Plants* **1993**, 1, 47–56.

Heath H.B.; Reineccius G.A. *Flavour chemistry and technology*. Macmillan Publishers, USA, 1986.

Hussien, M.S. Response of growth, yield and essential oil of coriander and dill to different nitrogen sources. *Egyptian J. Horticulture* **1995**, 22, 1–10.

Lewinsohn, E.; Gijzen, M.; Muzika, R.M.; Barton, K; Croteau, R. Oleoresins in grand fir (*Abies grandis*) saplings and mature trees. Modulation of this wound response by light and water stresses. *Plant Physiol.* **1993**, 101, 1021–1028.

Mallavarapu, G.R.; Kulkarni, R.N.; Baskaran, K.; Rao, L.; Ramesh, S. Influence of plant growth stage on the essential oil content and composition in davana (*Artemisia pallens* Wall.). *J. Agric. Food Chem.* **1999**, 47, 254–258.

McGarvey, D.J.; Croteau, R. Terpenoid metabolism. *Plant Cell* **1995**, 7, 1015–1026.

Muni Ram; Patra, D.D.; Sushil Kumar. Nitrogen fertilization of palmarosa in continuous cropping. *J. Herbs Spices Med. Plants* **1997**, 5, 53–61.

Munne-Bosch, S.; Alegre, L.; Schwarz, K. The formation of phenolic diterpenes in *Rosmarinus officinalis* L. under Mediterranean climate. *Eur. Food Res. Technol.* **2000**, 210, 263–267.

Perry, N.B.; Anderson, R.E.; Brennan, N.J.; Douglas, M.H.; Heaney, A.J.; McGimpsey, J.A.; Smallfield, B.M. Essential oils from Dalmatian sage (*Salvia officinalis* L.): variations among individuals, plant parts, seasons and sites. *J. Agric. Food Chem.* **1999**, 47, 2048–2054.

Rish, S.J.; Reineccius, G.A. Spray-dried orange oil: effect of emulsion size on flavour retention and shelf stability. In *Flavour encapsulation*, eds S.J. Rish, G.A. Reineccius. ACS symposium series 370; American Chemical Society: Washington, D.C., 1988; pp 67–77.

Sefidkon, F.; Dabiri, M.; Rahimi-Bidgoly, A. The effect of distillation methods and stage of plant growth on the essential oil content and composition of *Thymus kotschyanus* Boiss. & Hohen. *Flavour Fragrance J.* **1999**, 14, 405–408.

Senatore, F. Influence of harvesting time on yield and composition of the essential oil of a thyme (*Thymus pulegioides* L) growing wild in Campania (southern Italy). *J. Agric. Food Chem.* **1996**, 44, 1327–1332.

Trease, G.E.; Evans, W.C. *Pharmacognosy*. Bailliere Tindall, 1983.

Tyler, V.E.; Brady, L.R.; Robbers, J.E. *Pharmacognosy*. Philadelphia: Lea & Febiger, **1981**.

Waterman, P.G.; Mole, S. *Analysis of phenolic plant metabolites*. Blackwell, Oxford, 1994.

Young, S.L.; Sarda, X.; Rosenberg, M. Microencapsulating properties of whey proteins. 2. Combination of whey proteins with carbohydrates. *J. Dairy Sci.* **1993**, 76, 2878–2885.

SUMMARY

This thesis deals with research into the aromatic constituents of some plants of the families *Umbelliferae* and *Asteraceae*. The aim of the research was to elucidate the aroma composition of plants growing in Lithuania and to describe some aspects of flavour processing and release.

The composition of the aroma of the following plants was investigated: caraway, lovage, parsley, celery, dill and costmary. The aroma of these plants was analysed using various techniques, including gas chromatography combined with mass spectrometry and sniffing port detection (in the case of lovage). The investigation of the chemical composition of aromatic plants was focused on several factors influencing their aroma: such as harvesting time, anatomical part of plant, method used to isolate the volatiles, cultivar, and the fertilisers used in crop cultivation. The further processing and release of aromatic volatiles was also investigated. The properties of emulsions produced with essential oils were examined and the oil/water interface formed was characterised with regard to the adsorption of proteins and lipids. Encapsulated flavours were analysed and described. The ability of milk proteins to function well as encapsulating agents for essential oils was demonstrated in this study.

In **Chapter 1**, various aspects of flavour perception, the use of plants as a source of natural flavours, and the preservation of flavour by processing and encapsulation are discussed. Flavour isolation and analysis, and factors affecting flavour release, are briefly presented (including a literature review). The aims and objectives of the work are outlined at the end of the chapter.

An investigation into the chemical composition of the essential oils of locally grown parsley, celery, dill and caraway plants is described in **Chapter 2**. The results show that the amounts of the major constituents were ranked in the same order as described in the published data. The dill herb analysed could be assigned to chemotype II (dillapiole). The seed yield, essential oil content and chemical composition of four different caraway cultivars and the influence of fertilisers on these characteristics were also examined and described in this chapter. The investigation showed that total concentration of essential oil in the fruits varied from 1.9 to 4.3 ml 100 g^{-1}. The yield of caraway fruits grown in the experimental fields varied over a wide range (from 984 to 2673 kg ha^{-1}) depending on fertiliser content, cultivation area and the cultivar itself.

Chapter 3 deals with the analysis of essential oils from different plant parts (leaves, stems, flowers and seeds) of lovage (*Levisticum officinale* Koch.) at different stages of plant growth. It was shown that the seasonal changes in leaves were less noticeable than in the stems. Seeds and flowers possessed the highest yields of oil. Leaves exhibited their highest oil content before the stage of bud formation. α-Terpinyl acetate was found to be the predominant compound in leaves and stems (up to 70%), and β-phellandrene in seeds and flowers (61.5% and 40.8%, respectively); Z-ligustilide was confirmed as a major lovage phthalide, constituting from 4.4% to 11.7% of the essential oils in leaves and from 4.8% to 13.8% of the essential oils in the stems, depending on the time of harvesting.

In **Chapter 4,** a study of the essential oils from leaves and flowers of costmary, *Balsamita major* (L.) Desf. (syn. *Chrysanthemum balsamita* L.) at different plant growth stages showed that the highest oil content in both leaves and flowers is obtained before full flowering; 1.15% and 1.34% (w/w) respectively. Seventy-eight volatile compounds were identified in the oils of *Balsamita major*, of which 58 (19 tentatively, 39 positively) have not been reported previously in this plant. Carvone and α-thujone were found to be the predominant compounds. Seasonal variations in the oil compositions were negligible except for the initial phase (May 25), when the content of carvone was lower and the contents of α-thujone and sesquiterpenes higher. Absolute amounts of most components were highest during the bud-formation stage.

Flavour release measured by the dynamic headspace (DHS) method from different anatomical parts of lovage (leaves, stems, flowers, seeds and roots) at various plant growth stages is described in **Chapter 5**. Volatiles were isolated by DHS and characterised by GC–FID and GC–olfactometry (GC–O) techniques. Ninety-eight compounds were identified in the samples, 41 of which are reported among lovage volatiles for the first time. Qualitative differences in the composition of DHS constituents in various anatomical parts of the plants were not significant, whereas the quantities of a number of identified volatile compounds were different in leaves, stems, flowers, seeds and roots. Seasonal variations in the composition of headspace volatiles were also detected. Except for roots, β-phellandrene was found to be the most abundant headspace component in all anatomical parts of lovage.

The sniffing panel characterised effluents from a GC column and odour descriptors were attributed to the recognised constituents. None of the detected constituents was recognised as a lovage key aroma compound.

The stability and properties of oil-in-water emulsions with caraway and olive oils as dispersed phases were described in **Chapter 6.** It was shown that emulsions made with triglyceride oil are more stable than those made with caraway essential oil as the dispersed phase. Emulsion stability can be improved considerably by adding soyabean phosphatidylcholine (sb-PC) and increasing the protein concentration. Adsorption of the lipid from the oil and the protein from the aqueous phase at the oil/water interface can be studied by applying ellipsometry. Independently of the concentration used, close to monolayer coverage of sb-PC is reached at the caraway oil/aqueous interface. However, at the olive oil/aqueous interface, the presence of only a small amount of sb-PC leads to an exponential increase in the layer thickness with time beyond monolayer coverage. The amount of β-lactoglobulin adsorbed at the caraway oil/aqueous interface was the same as at the olive oil/aqueous interface and corresponded roughly to a protein monolayer.

Chapter 7 deals with the encapsulation of caraway essential oil into milk protein-based matrices. Essential oil of caraway can successfully be encapsulated by milk derivatives either alone or combined with carbohydrates. In a comparison between two milk derivatives, SMP and WPC, the latter exhibited better encapsulating properties. Partial replacement of WPC by carbohydrates increases the retention of volatiles during spray drying and enhances the protective properties of solidified capsules against oxidation and the release of volatiles during the timed period. SMP-based matrices showed the opposite tendency. The inner and outer structural features of spray-dried capsules indicated that good physical protection is afforded to the caraway essential oil.

The main results of the study are discussed in **Chapter 8**. It can be concluded that many factors affect the yield and composition of volatile compounds in plants: these include cultivar, climatic and agronomic conditions (fertilisation rate, cultivation site), harvesting time, anatomical part of the plant, and the method used to isolate and characterise volatiles. However, essential oils can be successfully processed by emulsifying and encapsulating them

in milk proteins, which helps to minimise losses from the oxidation of volatiles during storage and also improves handling of the flavouring material during technological applications.

SAMENVATTING

In dit proefschrift wordt een onderzoek beschreven naar de aromastoffen die voorkomen in enkele planten van de families *Umbelliferae* en *Asteraceae*. Het doel van het onderzoek was het ophelderen van de samenstelling van de aroma's van planten, die in Litouwen groeien, en het beschrijven van enkele aspecten van hun verwerking en de daarbij optredende verliezen.

De samenstelling van het aroma van de volgende planten werd onderzocht: karwij, maggi, peterselie, selderie, dille, balsemwormkruid. Het aroma van deze planten werd geanalyseerd met verschillende technieken, zoals gaschromatografie in combinatie met massaspectrometrie en snuffelpoort detectie (in het geval van de maggiplant). Het onderzoek naar de chemische samenstelling van het plantaardige aroma was gericht op verschillende factoren, zoals het moment van oogsten, het anatomische gedeelte van de plant, de gebruikte methode om de vluchtige verbindingen te isoleren, het ras en de kunstmestgift tijdens de groei. De verdere verwerking tot aroma en de daarbij optredende verliezen werd ook bestudeerd. Er werden emulsies gemaakt van essentiële oliën, waarvan de eigenschappen werden onderzocht, evenals de adsorptie van eiwitten en vetten aan het olie/ water grensvlak. Ook werden aromastoffen in capsules geanalyseerd en beschreven. In deze studie werd aangetoond dat melkeiwitten goed kunnen functioneren als grondstoffen voor capsules van essentiële oliën.

In **Hoofdstuk 1** worden verschillende aspecten besproken van de geur– en smaak-waarneming, evenals het gebruik van planten als bron van natuurlijk aroma en het behoud van aromastoffen tijdens verwerking (o.a. in capsules). In het literatuuroverzicht wordt kort ingegaan op isolatie en analyse van aroma en de factoren, die aromaverliezen beïnvloeden. De doelstellingen van de studie worden uiteengezet aan het einde van het hoofdstuk.

Een onderzoek naar de chemische samenstelling van de essentiële oliën van lokaal verbouwde gewassen van peterselie, selderie, dille en karwij wordt beschreven in **Hoofdstuk 2**. De resultaten laten zien dat de hoeveelheden van de belangrijkste bestanddelen in dezelfde volgorde en orde van grootte voorkwamen als beschreven in de literatuur. De geanalyseerde dille kon ondergebracht worden bij het chemotype II (dilleapiole). De zaadopbrengst, het essentiële oliegehalte en de chemische samenstelling van vier verschillende karwij-rassen, en de invloed van de kunstmestgift daarop, werden onderzocht en als zodanig beschreven in dit hoofdstuk. Het onderzoek toonde aan dat totale concentratie aan essentiële olie in de vruchten schommelde tussen 1,9 en 4,3 ml per 100 g. De opbrengst aan vruchten van de karwij, die op

de experimentele velden groeiden, gaf flinke verschillen te zien (van 84 kg tot 2673 kg per ha) in samenhang met de kunstmestgift, de standplaats tijdens de groei en het ras zelf.

Hoofdstuk 3 gaat over de analyse van essentiële oliën van verschillende delen van de maggi-plant (blad, stengel, bloem en zaad) bij verschillende stadia van de groei van de plant. Er werd aangetoond dat seizoensinvloeden minder duidelijk waren voor het blad dan voor de stengel. Zaden en bloemen gaven de hoogste opbrengsten aan olie. Het blad had het hoogste oliegehalte voor het stadium van de knopvorming. Er werd vastgesteld, dat α-terpinyl acetate de belangrijkste verbinding was van het blad en de stengel (tot 70%), en β-phellandreen van het zaad en de bloem (respectievelijk 61,5% en 40,8%). 2-Ligustilide werd ook in dit onderzoek gezien als een belangrijk ftalide van maggi, de essentiele olie van het blad bevatte 4,4 tot 11,7% en dat van de stengel 4,8 tot 13,8%, beide afhankelijk van het moment van oogsten.

In een onderzoek naar verschillende groeistadia **(Hoofdstuk 4)** werd aangetoond, dat de grootste hoeveelheid essentiele olie vlak voor de bloei van balsemwormkruid voorkwam, namelijk in het blad en de bloem respectievelijk 1,15% en 1,34%. De vluchtige verbindingen van de olie van balsemwormkruid werden geïdentificeerd, waarvan er 58 (19 voorlopig en 39 definitief) niet eerder waren gerapporteerd over deze plant. Carvon en α-thujon waren de belangrijkste verbindingen. Seizoensinvloeden op de oliesamenstelling waren te verwaarlozen behalve voor de beginfase (25 mei), toen de gehalten van α-thujon en sesquiterpenen hoger en het carvongehalte lager was. De absolute hoeveelheden van de meeste verbindingen waren het hoogst tijdens de knopvorming.

Het vrijmaken van aromastoffen met behulp van de dynamische headspace methode (DHS) uit verschillende anatomische delen van de maggiplant (blad, stengel, bloem, zaad en wortel) en bij verschillende groeistadia van de plant wordt beschreven in **Hoofdstuk 5**. Vluchtige verbindingen worden geïsoleerd met behulp van DHS en nader gespecificeerd met GC-FID en GC-olfactometrische technieken. In de monsters werden 98 verbindingen geïdentificeerd, waarvan er 41 voor het eerst als verbindingen van de maggiplant worden gerapporteerd. Kwalitatieve verschillen in de samenstelling van de DHS isolaten van verschillende anatomische delen van de planten waren niet significant. Terwijl er wel verschillen gevonden waren voor de hoeveelheden van een aantal geïdentificeerde vluchtige verbindingen van het

blad, de stengel, de bloem, het zaad en de wortel. Seizoensinvloeden op de samenstelling van de DHS isolaten werden ook waargenomen. Behalve bij de wortel was β-phellandreen de meest voorkomende DHS verbinding in alle anatomische delen van de maggiplant. Het snuffelpanel gaf een beschrijving van de vrijkomende gassen van een monster op een GC kolom en kende attributen toe aan de bekende geurstoffen. Geen enkele hiervan werd herkend als een kenmerkende geur van de maggiplant.

De stabiliteit en andere eigenschappen van olie-in-water emulsies met karwij en olijfolie als gedispergeerde fase worden beschreven in **Hoofdstuk 6**. Er werd aangetoond dat emulsies met een triglyceride olie als gedispergeerde fase stabieler zijn dan die met een karwij essentiële olie. Emulsie stabiliteit kan aanzienlijk verbeterd worden door toevoeging van soja fosfatidylcholine (SFC) en door verhoging van de eiwitconcentratie. De adsorptie van lipiden uit de oliefase en van eiwit uit de waterfase aan het olie/ water grensvlak kan bestudeerd worden met behulp van ellipsometrie. Vrijwel een monolaag bedekking van het karwij olie/ water grenslak werd verkregen met SFC, en wel onafhankelijk van de gebruikte concentratie. Echter, bij het olijfolie/ water grensvlak, gaf de aanwezigheid van een klein beetje SFC gedurende een bepaalde tijd een exponentiele toename van de laagdikte bovenop de monolaag bedekking. De hoeveelheid β-lactoglobuline, dat adsorbeerde aan het karwij olie/ water grensvlak was hetzelfde als dat aan het olijfolie/ water grensvlak, en dat kwam globaal overeen met een eiwit monolaag.

In **Hoofdstuk 7** worden de karwij essentiële olie capsules gemaakt van matrixen van melkeiwit. De essentiële olie van karwij kan met succes in capsules van melkpreparaten worden opgenomen, zowel op zich als in combinatie met koolhydraten. In een vergelijking van 2 melkpreparaten, met name een magere-melk eiwit en een weieiwit concentraat, bleken de capsules van laatstgenoemde de betere eigenschappen te bezitten. Gedeeltelijke vervanging van het weieiwit concentraat door koolhydraten verhoogt de retentie van vluchtige stoffen tijdens het sproeidrogen en wordt door de vaste stof van de capsules de beschermende werking (tegen aromaverliezen) voor een bepaalde tijd verhoogt. Matrixen van magere-melk eiwitten lieten een tegengestelde tendens zien. De opbouw van de binnen- en buiten-struktuur van de gesproeidroogde capsules toonde aan, dat een goede fysische bescherming van de essentiële olie van karwij mogelijk moest zijn.

De belangrijkste resultaten van de studie worden ter discussie gesteld in **Hoofdstuk 8**. De conclusie is, dat veel factoren de opbrengst en samenstelling van vluchtige componenten in planten beïnvloeden Deze zijn het ras, het klimaat en de landbouwkundige omstandigheden (kunstmestgift, plaats van de groei), het moment van oogsten, het anatomische deel van de plant, de gebruikte methode voor de isolatie en de analyse van de vluchtige stoffen. Essentiële oliën kunnen met succes verwerkt worden door emulgatie met melkeiwitten om er daarna capsules van te maken. Dit laatste helpt bij het tegengaan van verliezen door oxidatie van het aroma tijdens opslag en ook bij het gemakkelijker hanteerbaar maken van aromapreparaten voor technologische toepassingen.

ACKNOWLEDGEMENTS

List of publications

Bylaitė, E.; Venskutonis, R.; Roozen, J.P. and Posthumus, M.A. (2000). "Composition of essential oil of costmary [*Balsamita major* (L.) Desf.] at different growth phases." *J. Agric. Food Chem.,* in press.

Bylaitė, E.; Venskutonis, R. and Maždžierienė, R. (2000). "Properties of encapsulated caraway essential oil into milk proteins based matrices". *J. Agric. Food Chem.,*submitted.

Bylaitė, E.; Nylander, T. and Venskutonis, R. (1999). "Emulsification of caraway essential oil in water by lecithin and β-lactoglobulin – emulsion stability and properties the formed oil-aqueous interface". *J. Dairy Science,* submitted.

Bylaitė, E.; Roozen, J.P.; Legger, A.; Venskutonis, R.P. and Posthumus, M.A. (1999). "Dynamic headspace–gas chromatography–olfactometry analysis of different anatomical parts of Lovage (*Levisticum Officinale* Koch.) at eight growth phases." *J. Agric. Food Chem.,* submitted.

Venskutonis, R.P.; Kvietkauskaitė, D.; Bylaitė, E. and Šuliauskas, A. (1999). "Characterization of caraway (*Carum Carvi* L.) cultivated in Lithuania". *Horticulture and Vegetable Growing.* Scientific Works of the Lithuanian Institute of Horticulture and Lithuanian University of Agriculture, **18** (3), 85–92.

Bylaitė, E.; Venskutonis, R.P.; and Roozen, J.R. (1998). "Influence of harvesting time on the composition of volatile components in different anatomical parts of lovage (*Levisticum officinale* Koch.)". *J. Agric. Food Chem..* **46** (9), 3735-3740.

Bylaitė, E. and Venskutonis, R.P. (1997). "Stability of microencapsulated caraway (*Carum carvi* L.) essential oil during storage". In Proceedings of the 3rd main meeting of   EC Copernicus Concerted Action *"Process optimisation and minimal processing of foods",* Vol. 3 "Drying", eds. J.C.Oliveira and F.A.R. Oliveira, pp. 81-87.

Bylaitė, E.; Legger, A.; Roozen, J.P. and Venskutonis, R.P. (1996). "Dynamic headspace gas chromatography of different botanical parts of lovage (*Levisticum officinale* Koch.)". In

"Flavour Science: Recent developments", eds. A.J.Taylor, D.S.Mottram, The Royal Society of Chemistry, UK, p.66-69.

Venskutonis, R.P.; Bylaitė, E. and Narkevičius, R. (1996). "Optimisation of Encapsulation Conditions of Caraway *(Carum carvi* L.) essential oil". In Proceedings of the 2nd main meeting of EC Copernicus Concerted Action *"Process optimisation and minimal processing of foods"*, Vol. 3 "Drying", ed. J.C.Oliveira, pp. 95-101.

Bylaitė, E. and Venskutonis, R. (1994). "Essential oils of parsley and celery from Lithuania". Scientific works "Food chemistry and technology", Vilnius, Academia, p. 69-74.

Bylaitė, E. and Venskutonis, R. (1994). "Composition of commercial peppermint (Mentha piperita) essential oil from Lithuania". Proceedings of the conference "Maisto chemija ir technologija". Kaunas, "Technologija", p. 12-14

Bylaitė E., Venskutonis, R. (1994). "Volatile constituents of dill". Proceedings of the conference "Maisto chemija ir technologija". Kaunas, "Technologija", p. 15-18.

CURRICULUM VITAE

Eglė Bylaitė was born on 22 July, 1969 in Birštonas, Lithuania. In 1987 she obtained high school diploma in Raseiniai and started her studies of Food Technology at Kaunas University of Technology. During these studies she carried out research at the Department of Organic Chemistry, Gent University, Belgium which was sponsored by Tempus program and in 1993 graduated with an MSc in Food Science. From 1995 she was a doctoral student of the cooperative PhD program between laboratories of Food Science of Kaunas University of Technology, Lithuania and Wageningen University, The Netherlands. In the period of 1995/1996 she was working at the Department of Agrotechnology and Food Sciences, Wageningen University. Later research work was continued at the Department of Food Technology, Kaunas University of Technology. In 1998 Eglė carried out research at the Department Physical Chemistry 1, Lund University, Sweden sponsored by Nordic Academy for Advanced Studies (NorFA). Since Jan 2000 she stayed at the Department of the Agrotechnology and Food Sciences for the completing of her PhD thesis by writing this book. All research that has taken place during her doctoral studies is described in this thesis.