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by

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The pure oil of flowers, their naked perfume, one hundred thousand times concentrated to obtain some drops of absolute essence. The odour of this essence was not pleasant at all: it was a strong and intense odour, almost painful. And yet, it was enough to pour one drop in a liter of alcohol to bring it into a new life and revive a whole field of flowers.

Patrick Söskind, *in Perfume: The story of a murderer*

RESUMO

O sentido do olfacto tem sido alvo de um interesse crescente por parte da comunidade científica e pela sociedade, afectando consideravelmente o comportamento das pessoas uma vez que está intimamente ligado às emoções e à memória. A indústria de aromas e fragrâncias oferece a possibilidade de se viver novas experiências sensitivas através da criação de novos cheiros e paladares. A Ciência e a Engenharia têm aqui um papel importante, na medida em que é necessário sintetizar novas moléculas, extrair os aromas e fragrâncias das plantas e misturar os materiais de forma a que o resultado seja um aroma ou fragrância agradável, que tenham o desempenho pretendido.

Nesta tese pretende-se considerar as duas principais áreas da indústria de fragrâncias e aromas, com especial destaque para a perfumaria: a produção de matérias-primas naturais para perfumes e a optimização da composição do perfume. O objectivo é usar recursos naturais e nacionais e tecnologias limpas para produzir matérias-primas para perfumes e modelização e simulação matemática para prever o odor de uma mistura de perfume e a sua evolução durante a evaporação.

Assim, o gerânio-rosa *(Pelargonium sp)* e a esteva (*Cistus ladanifer*) foram estudados, tendo em conta que são plantas bem adaptadas ao território português e que podem ser cultivadas em grandes extensões para produzir matérias-primas importantes para perfumes como notas intermédias e de base, respectivamente. Em seguida, a extracção supercrítica foi aplicada à extracção do gerânio-rosa para se obter um extracto de grande qualidade, totalmente isento de resíduos de solvente. A desterpenação de óleos essenciais foi também estudada no sentido de se obterem produtos de alto valor acrescentado.

Uma metodologia inovadora baseada no conceito de *Perfumery Ternary Diagrams* e *Odour Value* foi usada para prever o cheiro de uma mistura de perfume e assim reduzir as experiências necessárias para fazer a composição de um perfume. Esta metodologia foi validada experimentalmente através da medição de valores de *odour threshold* e análises de *headspace*. Um modelo difusional foi usado para simular a evaporação de um perfume e avaliar quantitativamente o desempenho em termos de odor através dos parâmetros: impacto, difusão, tenacidade e volume.

ABSTRACT

The sense of smell is being rediscovered by science and society, having a very powerful effect on people since it is strongly connected with the emotional level and memory. The Flavour & Fragrance industry is the one able to give us new sensory experiences by delivering new scents and tastes. Science and Engineering has an important role here since it is necessary to synthesize these new molecules, extract the scents from the plants and blend the materials in such a way that it results in a pleasant flavour or fragrance and that performs as expected.

This thesis aims to cover the two main areas of F&F industry, with special focus on perfumery: the production of natural perfumery raw materials and the optimisation of the perfume mixture. The objective is to use natural national resources, clean technologies to produce perfume materials and mathematical modelling and simulation to predict the odour of the perfume composition and its evolution during evaporation.

In view of this, rose geranium (*Pelargonium sp*) and rockrose (*Cistus ladanifer*) were studied since they are well adapted to Portuguese territory and they can be cultivated in large extensions to produce important perfumery materials as middle and base notes, respectively. Afterwards, the supercritical fluid extraction (SFE) was applied to the extraction of rose geranium to get a high quality extract, completely free of solvent residues. The deterpenation of essential oils using adsorption chromatography was also studied in order to produce a high-added value product.

A new methodology based on Perfumery Ternary Diagrams (PTD) concept and Odour Value was used to predict the smell of a perfume mixture and this way reduce the tedious experimental trials necessary to make a perfume composition. The PTD methodology was validated experimentally by measuring odour threshold values and headspace analysis. A diffusion model was used to simulate the evaporation of a perfume and evaluate quantitatively the odour performance parameters, namely: impact, diffusion, tenacity and volume.

RÉSUMÉ

Le sens olfactif trouve un intérêt croissant auprès de la communauté scientifique et la société d'une manière générale, affectant considérablement le comportement des personnes lorsqu'il est lié aux émotions et à la mémoire. L'industrie des arômes et des fragrances nous offre la possibilité de vivre des expériences sensitives inédites à travers la création de nouveaux goûts et parfums. La science et l'ingénierie ont ici un rôle important depuis qu'il est nécessaire de synthétiser de nouvelles molécules, d'extraire des senteurs à partir de plantes et de mélanger des matériaux de manière à obtenir un arôme ou une fragrance agréable.

Cette thèse a donc pour objectif de recouvrir les deux principaux domaines de l'industrie des fragrances et des arômes, avec un intérêt particulier pour la parfumerie : la production des matières premières naturelles pour les parfums et l'optimisation de la composition d'un parfum. Le but est d'utiliser les ressources naturelles nationales et les technologies propres pour produire les matières premières des parfums, puis la modélisation et la simulation mathématique afin de prédire l'odeur de la composition d'un parfum et son évolution durant l'évaporation.

Ainsi le géranium rose *(Pelargonium sp)* et le ciste lédon (*Cistus ladanifer*) furent étudiés, dans la mesure où il s'agit de plantes particulièrement bien adaptées au territoire portugais et qui peuvent être cultivées d'une manière extensive afin de produire les matières premières importantes des parfums, respectivement comme note de coeur et note de found. Ensuite une extraction supercritique fut appliquée à l'extraction du géranium rose pour obtenir un extrait de haute qualité, totalement dépourvu de résidu de solvant. La déterpénation des huiles essentielles fut également étudiée afin d'obtenir des produits à haute valeur ajoutée.

Une méthodologie innovante basée sur le concept de *Perfumery Ternary Diagrams* et *Odour Value* fut utilisée pour prédire la senteur d'un mélange de parfums et ainsi réduire le nombre d'expériences nécessaires pour élaborer la composition d'un parfum. Cette méthodologie fut validée expérimentalement à travers des mesures de *odour threshold* et des analyses *headspace*. Un modèle de diffusion fut utilisé pour simuler l'évaporation d'un parfum et évaluer quantitativement les paramètres de performances odorantes : impact, diffusion, ténacité et volume.

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CHAPTER 1

INTRODUCTION

1.1 Relevance and Motivation

The Flavour & Fragrance (F&F) industry offers a wide range of products that participate in our everyday life in several forms, from flavours for food and beverages, to perfumes, skin deodorants, bath gels, air freshners, detergents, perfumed cards, candles, and many others. The F&F covers a wide range of production scales and product costs, producing two kinds of products: (1) raw materials consisting of a few tons of valuable raw materials like the natural products and 5000-6000 tons of synthetic ingredients with costs comparable to bulk chemicals; (2) flavour blends and fine fragrances with high-added value.

Our society is very visual-orientated. However, the sense of smell is very powerful since it triggers emotions and memories, being intimately related with the feeling of wellbeing and pleasantness, influencing the mood and the behaviour at the unconscious level. Marketing has been using this power to promote their products, by making them more attractive to the consumer because they smell nice, make people feel good (like taking bath and making aromatheraphy at the same time) or by enhancing the functional benefit of the product like imparting the smell of clean after washing clothes with a detergent or softner. In particular, perfumes or fine fragrances are aimed as a luxury object that expresses the personality or sensuality of the wearer and at the same time is a fashion accessory.

The return to nature is a growing trend today, because people believe that natural is good and safe. Therefore the demand for natural ingredients and natural-based products is increasing, including for the F&F industry.

Portugal has a Mediterranean climate and a good soil favourable for the growth of aromatic plants that could be used to produce essential oils and extracts for perfumery and flavours for food and pharmaceutical industry. Therefore there is an opportunity for taking advantage of these natural resources and give rise to alternative cultivations that could turn the agriculture activity more profitable and more attractive, especially in the areas in the Interior Region that are in risk of desertification.

On the other hand, the use of environmentally friendly or clean technologies is encouraged due to regulatory constraints, more and more strict, and due to social-economical-political pressures that result from people's awareness and concern about environmental issues, namely pollution and green-house effect. Supercritical carbon dioxide technology is a clean technology that can be used for extraction or fractionation of natural extracts from plants, having the advantages of not leaving any residue in the natural product, using as a solvent a non-toxic and inexpensive gas – carbon dioxide (CO2). Therefore CO2 becomes a raw material instead of being a waste emission, having the advantages of being low energy-consuming since the operating temperatures are lower and yielding a natural ingredient with superior organoleptic quality, i.e., the odour more resembling of the natural source.

The other F&F field is the manufacture of flavour and fragrance blends. The making of a perfume mixture – compounding - involves a considerable know-how that enables to choose and to blend in such a way that the fragrance performs as it is required by an initial costumer brief. This work to date has still a strong empirical background, relying mainly on trial-and-error. The scientific work that enables optimization is unpublished due to competition and secrecy inherent to this industry. The time pressure and cost constrains, due to increasingly short submission deadlines for new products and high competition, demands for a more efficient approach that is able to reduce the extensive trial-and-error just to a few experiments and optimize the formulation to comply with the cost limits. It is necessary to be able to predict the behaviour of fragrance performance, through modelling the physical phenomena involved in the evaporation and diffusion of perfumes. And here researchers can give an important contribution. That was the main motivation of this thesis.

1.2 Objectives and Outline

The general objective of this Ph. D. thesis is to design a perfume with a scientific basis, making use of Portuguese natural resources, clean technologies and mathematical modelling. The scope of this work comprises all the important tasks in the perfumery industry, from the screening and characterization of perfumery raw materials, through the production of natural raw materials at a laboratory-scale and at a pilot-scale using carbon dioxide supercritical fluid extraction as clean technology, adding value to natural materials by deterpenation using preparative liquid chromatography to fractionate essential oils, and finally creating perfumes based on a systematic approach of measuring important data in perfumery (odour threshold values, headspace concentrations) and predicting perfume behaviour by modelling evaporation and diffusion, instead of using the common practice of trial-and-error to find a perfume formulation (usually in the liquid form) having the desired performance in the gas phase (after evaporation). The GC/MS technique has been a fundamental tool that has supported all the work, which enabled the building of an extensive database of information based on the characterization of the natural materials produced and all the raw materials collection, the study of the composition of commercial perfumes and of our perfumes and the measurement of headspace concentrations. The olfactometer equipment was also essential in this work to obtain the sensory data of odour thresholds.

This thesis is organized in nine chapters.

In Chapter 2, a review is made, covering several aspects related with the flavour and fragrance industry and its applications, focusing on the perfumery area. Firstly, the economic context of F&F industry is presented, followed by a brief description of the sense of smell and the regulatory issues. The perfumery raw materials and their production are described, considering supercritical fluid extraction and deterpenation of essential oil in more detail. The state-of–the-art in composing and manufacturing perfumes is presented and finally the scientific and technical contribution given to the perfumer work is shown.

Chapter 3 describes the screening and characterization of natural resources that can be used to produce raw materials for perfumery. Two Portuguese plants are studied – rose geranium (*Pelargonium sp.*) and rockrose (*Cistus ladanifer*). Geranium oil is one of the most marketed essential oil, being used largely as middle note in perfumes due to its rose scent, replacing in many cases the very expensive rose oil. On the other hand, rockrose grows widely all over the country and is the raw material for producing Labdanum and cistus oil, two perfumery materials used in perfumes to impart an amber leathery odour. Both plants are well adapted to Portugal territory, with high potential of being exploited as a natural resource for the production of naturals.

In Chapter 4 the carbon dioxide supercritical fluid extraction (SFE) is applied to the production of natural extract of rose geranium. The SFE equipment was designed and constructed in this laboratory, in order to have a more flexible operation and to allow the use of higher quantities of plant.

In Chapter 5, it is considered preparative liquid chromatography to fractionate essential oils, using silica particles as adsorbent. This process was used to remove the monoterpene fraction of lemon oil, since these are unstable and have low solubility in alcohol. The separation was optimized by simulation, using a model developed in the scope of this project.

In Chapter 6, a new methodology for compounding perfumes is shown, based on the new concept of Perfumery Ternary Diagram (PTD). The PTD methodology predicts the odour value (related with the odour intensity) of a perfume mixture, giving some guidance to the perfumer about the expected smell and reducing this way the number of trials to find the right composition.

In Chapter 7, the methodology of Perfumery Ternary Diagram is validated by comparing the predicted odour values with the actual odour values of a perfume test mixture, based on the experimental determination of odour threshold values and headspace concentrations.

In Chapter 8, the odour performance of perfumes is assessed by predicting the behaviour of perfume mixtures over time and space by using a diffusion model, considering the non-idealities in the liquid phase. This way it is possible to evaluate quantitatively the odour performance parameters, namely impact, tenacity, diffusivity and volume, by contrast with the currently used qualitative procedure based on the perfumer subjective evaluation.

Finally, in Chapter 9 the main conclusions drawn from this thesis are presented, together with the suggestions for future work.

CHAPTER 2

PERFUMERY – A REVIEW

In this chapter, initially a general overview on flavour & fragrance (F&F) field is given, with highlight to F&F industry and its applications, regulatory issues and a brief description of the olfactory sense. Thereafter, a description of the main parts involved in perfume production, from the raw materials to the final product – perfume – is presented in some detail.

First, a description and classification of the different kind of materials normally used in perfumery is shown, giving some highlight to the differences between naturals and synthetics. The production of natural raw materials in Portugal is also described.

Then, the different industrial methods of production of natural perfumery materials is reviewed as well as processes used to add-value to natural essential oils.

Finally, the final process of production of perfumes is reviewed, namely the classification, formulation, compounding and manufacture, as well as a detailed highlight to the scientific basis of perfumery used nowadays in order to give a systematic approach to the creative art of perfumery.

2.1 The F&F Industry

The flavour and fragrance (F&F) industry is a very important economic sector, corresponding to worldwide estimated sales market of 16 billion dollars in 2003 (Leffingwell&Associates, 2004). The F&F industry is intermediary between the pharmaceutical and bulk chemicals industries, with a scale of production closer to the pharmaceutical products but the cost per kg of product is in between the pharmaceuticals and the bulk chemicals from petrochemical industry (Pybus and Sell, 1999).

The worldwide market is controlled by ten main F&F companies, concentrated in Europe, USA and Japan (see Figure 2.1), which altogether represent 65% of share market, as is shown in Figure 2.2 (Leffingwell&Associates, 2004). The Western Europe and North America are the main markets and the most mature ones, accounting for 60% of total demand (Short, 2002), while Asia and Latin America are growing markets (Figure 2.3).

Figure 2.1 Worldwide geographical distribution of top 10 F&F companies.

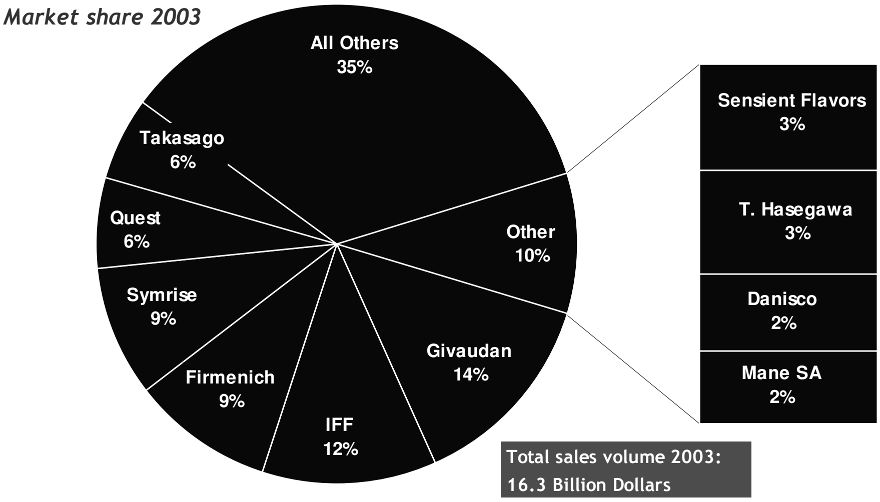


Figure 2.2 Market share of Flavour & Fragrance Industries in 2003 (source: Leffingwell & Associates, in Leffingwell, 2004).

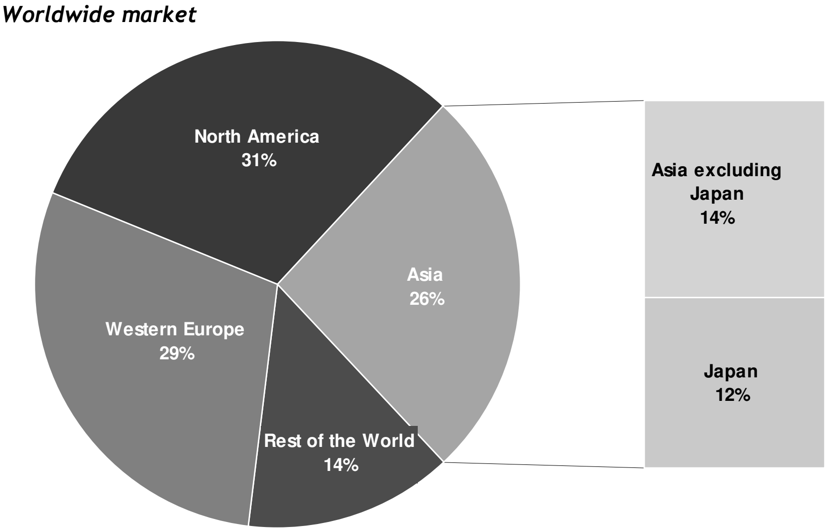


Figure 2.3 The worldwide market of F&Fin 2002 (source: Freedonia Group and C&EN estimates, in Short, 2002).

F&F activity is divided in four main categories, as can be seen in Figure 2.4: the production of raw materials – naturals and aroma chemicals- and blends of flavours and fragrances. The last two categories constitute the core of the business with 76% of the sales volume. The F&F have a wide range of applications, namely fine fragrances and perfumes, cosmetics, soaps, toiletries, household products, diffusers and candles, aromatherapy, as well as flavours for the food and pharmaceutical industries (see Figure 2.5).

In cosmetics, toiletries and household products, fragrances are used with the purpose of influencing the consumer to buy the product, either by enhancing its benefit, signal the product to be easily recognizable or simply to be pleasant and call the attention of the consumer.

It is usual that the biggest F&F companies are simultaneously producers, suppliers and clients of the other companies in the business, i.e., the companies are competitors and clients at the same time. There are others that specialise only in blending fragrances and flavours for other companies, buying all the ingredients. The distribution, marketing and publicity to promote the products constitute the last links of the value chain.

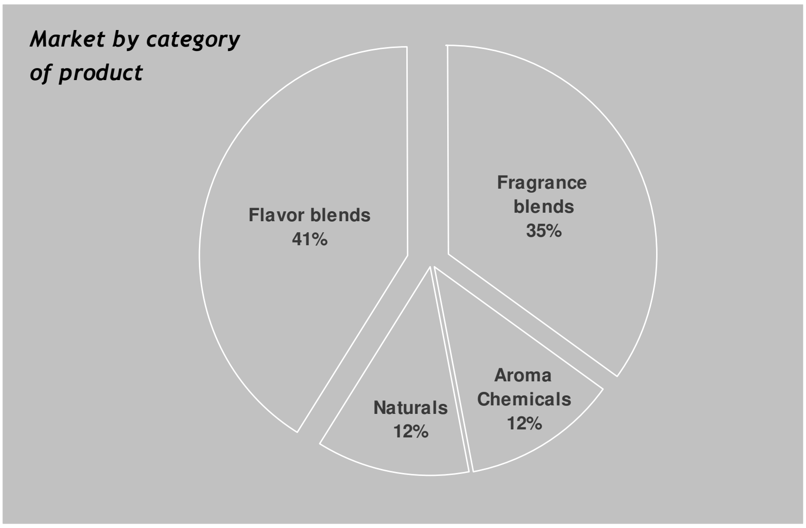
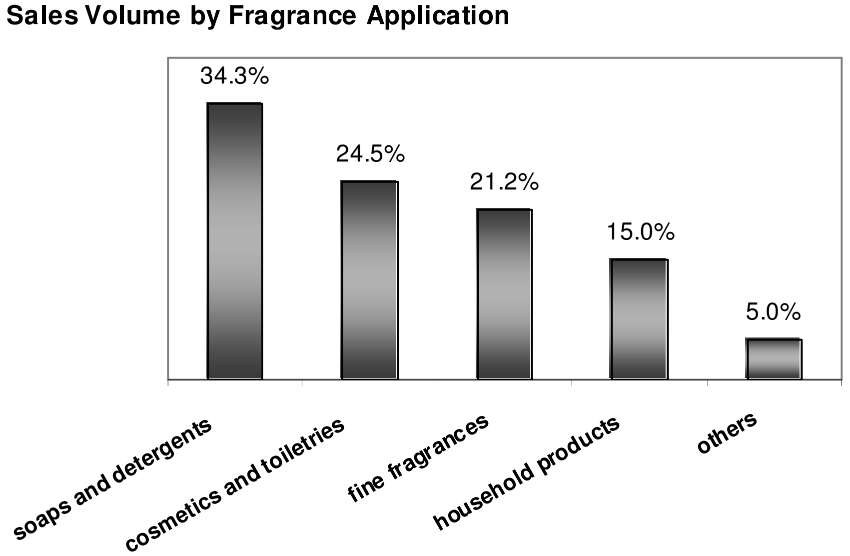


Figure 2.4 Market of F&F industry by category of product in 2002 (source: Freedonia Group and C&EN estimate, in Short, 2002).

(a)



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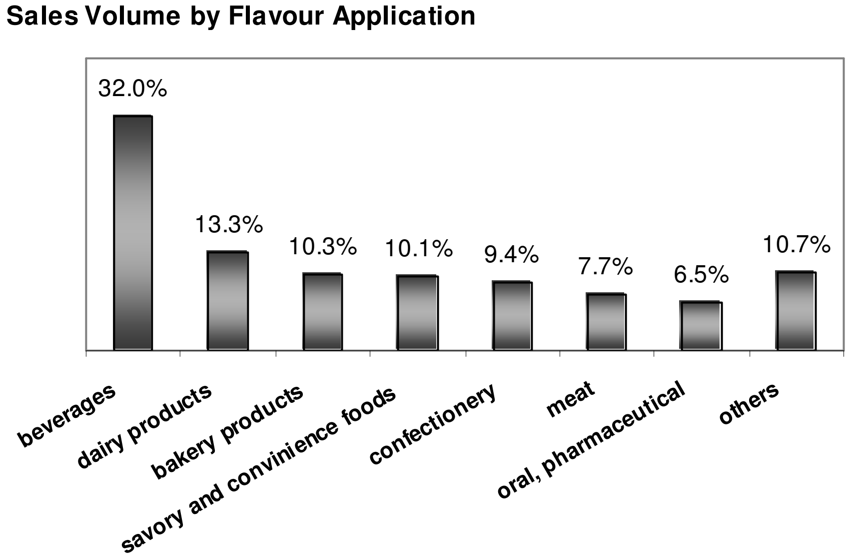


Figure 2.5 Sales volume of F&F products by application: (a) Fragrances; (b) Flavours (source: Leffingwell & Associates (2002) and IAL consultants (2001) in Rouhi, 2003a).

The perfumes are commercialized under a name of a designer or an individuality, but the perfume making is outsourced to F&F companies, which identity and the name of the perfumer involved is not disclosed for commercial reasons. For example, Calvin Klein has perfumes made by Firmenich (CK One, Truth), Givaudan (Obsession, Contradiction), IFF (Eternity) and Mane (Escape).

The companies by keeping the formula secret instead of patenting hope to prevent the diffusion of counterfeit copies of their perfumes and "the trickle-down effect" of matching a successful fine fragrance for application in lower quality products for mass market, personal care and household products. Therefore, in the perfume label it is just mentioned "fragrance" or "perfume", and this way the secrecy is somewhat protected. However, with the development of the new techniques of gas chromatography and mass spectrometry (GC/MS), the composition of the fragrance could be disclosed and a boom of copies has occurred. Moreover, the GC/MS analysis of natural products allows the production of synthetic materials with similar characteristics, at a considerably lower cost.

Seeking for new ingredients is a way of F&F industry to get competitive advantage by offering a new scent and to protect their products by patenting that component and using it exclusively in their formulations.

Another driving-force for F&F industry is the need to add value to the products by supplying multifunctional fragrances: besides perfuming the product, the fragrance should have additional properties, such as antibacterial activity or insect repellence (Rouhi, 1999).

The market is hence much more competitive and constantly dealing with new challenges in order to keep a forward-looking attitude/strategy (PRODAROM, 2004):

- produce new raw materials;
- use or adapt new technologies for production;
- create new olfactory accords;
- find new clients or markets;
- expand the offer of products to other applications, like: household products, air fresheners, insecticides, paints;
- comply with increasing constraints for quality and safety use.

For the success of the new product launch, the teamwork of creators, researchers and marketing specialists is crucial for facing the competition, to meet deadlines and accomplish consistency between the fragrance concept, packaging and marketing.

The production of raw materials is another field of F&F industry. Naturals are obtained through plantations spread all over the world, mainly in the poor countries, having the production facilities close to the plant fields since most of the plant materials must be extracted shortly after harvesting, and frequently involving handpick of the flowers, as is the case of ylang-ylang and jasmine (Figure 2.6). On the other hand, some developed countries also cultivate aromatic plants, especially when the harvest can be mechanical, as is the case of the great extension of fields of lavender in France, in Grasse region (Figure 2.7).

The price of essential oils and plant extracts is established according to the concentration in the plant, the extraction process and manpower cost, being as much expensive as lower are the yields. There is a great variability in supply and prices of naturals depending on their availability, which also depends on the cultivation and/or harvest results of that year and on the increase or decrease in demand of a certain ingredient.

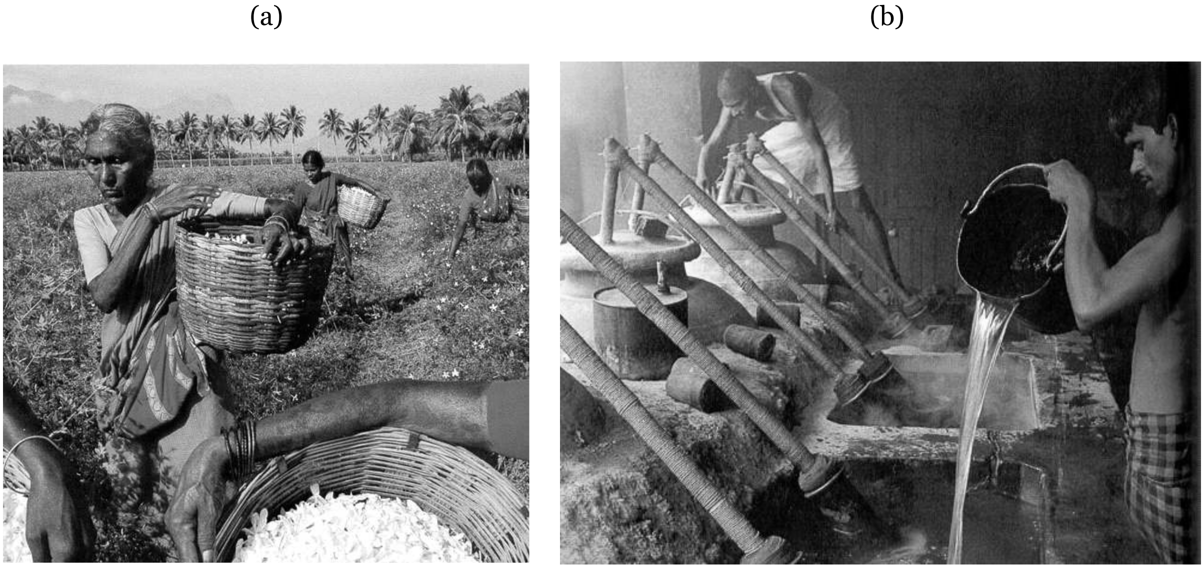


Figure 2.6 Production of naturals in poor countries: (a) harvest of jasmine flowers by handpick; (b) distillation using rudimentary equipment (source: Newman, 1998).

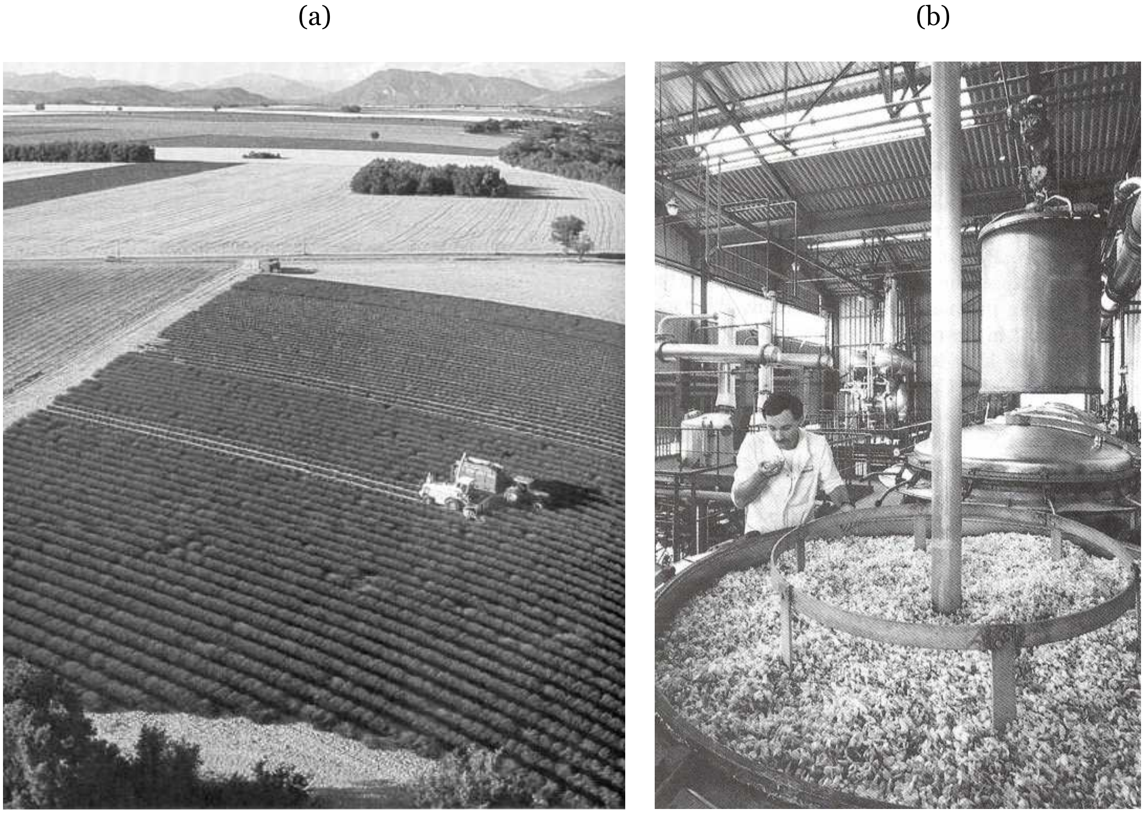


Figure 2.7 Cultivation fields of lavender and distillation unit, in Grasse, France (source:a- Newman, 1998; b- Pybus and Sell, 1999).

The naturals are mostly used in fine fragrances due to their high cost, which sometimes is even higher than gold price like tuberose absolute (20k€/kg) or iris root oil (25k€/kg). However due to environmental concerns and restricting health regulations, as well as the consumers are more aware of and concerned about these matters, the growth in demand for naturals will outpace that for aroma chemicals over the next years (Short, 2002).

F&F in Portugal

In Portugal, the imports of perfumery raw materials, perfumery products and cosmetics have grown 79%, and the exports increased 58%, in the period 1995 to 2002, according to INE (Instituto Nacional de Estatística) data. When considering the perfumes and eau de toilettes, the imports increased 24% but the exports decreased 52%, as can be seen in Figure 2.8. This means that the consumption of fragrances in Portugal had an average annual growth of 3%, having in 2002 an annual consumption per capita of 260 ml, which represents around 5 perfume bottles of 50 ml. Therefore, it is considered that there is a business opportunity for production of perfumes in Portugal.

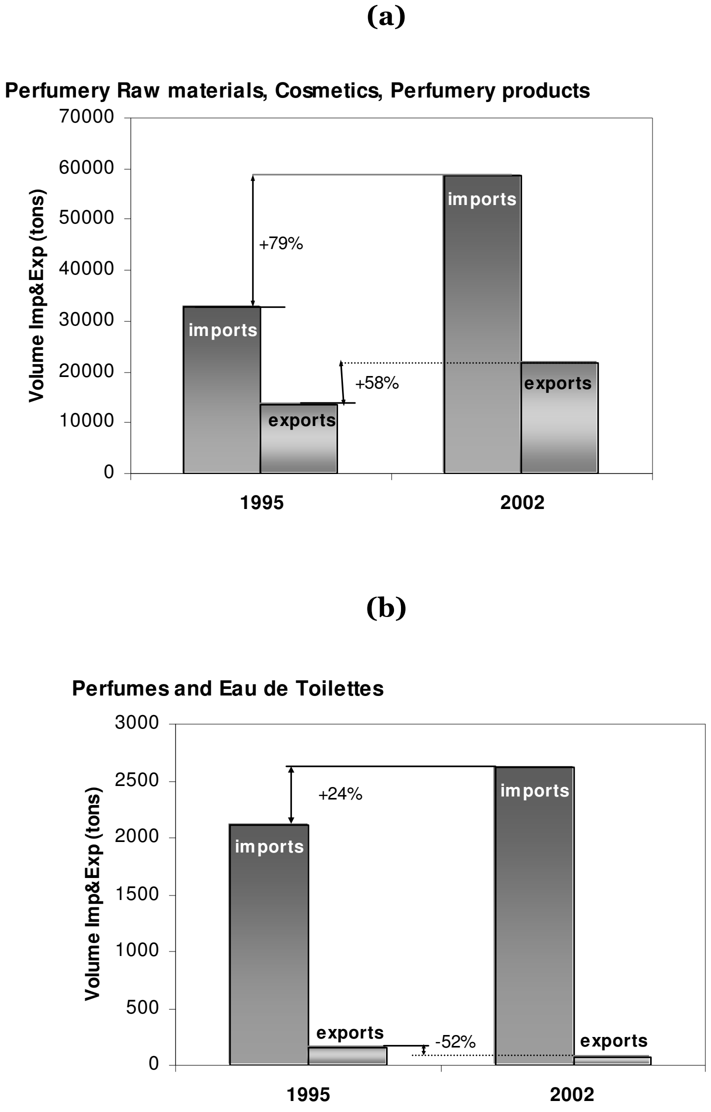


Figure 2.8 Volume of Imports and Exports in Portugal, for 1995 and 2002: (a) Essential oils, Resinoids, Cosmetics and Perfumery products; (b) Perfumes and Eau de Toilettes (source: INEl, 2002).

2.2 The regulatory context

Perfumery industry has been to date self-regulated, unlike the case of the food industry, by two major international organizations: International Fragrance Association (IFRA) and Research Institute for Fragrance Materials (RIFM). Many of the constraints that apply to chemical industry are also applicable to this industry.

IFRA represents over 100 manufacturers in 15 countries (Pybus and Sell, 1999). It has been created to serve the interests of the international fragrance industry and to protect the health of employees and consumers, by implementing a Code of Practice and setting product safety guidelines to be applied worldwide.

The Code of Practice (1975) comprises good manufacturing practices related with personnel, hygiene, storage, production, labelling, advertising, packaging and quality control. The use of fragrances must comply with the minimum safety standards, or if it has been in-use for a long time that there is no adverse reactions reported (PRODAROM, 2004; Pybus and Sell, 1999).

RIFM is an independent institution that evaluates the safety of fragrance ingredients and gathers information from research, literature and manufacturers, so that IFRA can establish the standards.

The evaluation tests include oral toxicity, skin irritation, skin sensitisation and photo-toxicity. The results are submitted to an independent panel of international experts in toxicology, pharmacology and dermatology and published as RIFM monographs in the journal “Food & Chemical Toxicology”. The risk of inhalation and ingestion are little significant under normal-use conditions due to the low concentrations involved (less than 2.5 ppm) (Pybus and Sell, 1999).

IFRA restrictions may be quantitative limitations expressed as concentrations for all or just some applications, being applied to 23 naturals and 61 synthetic ingredients (IFRA, 2004). The recommendations are to not use 32 ingredients, limit the use of 36 materials, 11 should be used if produced in certain purity criteria and manufacturing methods and 5 must be used together with other substances (PRODAROM, 2004). However, if the restrained component is a constituent of some natural material, it can be used if the natural material has been reported as safe. This happens, for example, with limonene, which as an aroma chemical is more restricted than as lemon oil although this oil has 95% of limonene.

2.3 The sense of smell

The sense of smell is intimately related to emotions and memory, influencing therefore significantly the human behaviour and mood. However, it is not essential for human survival as is for other animals, and probably because of that it is the less studied sense. In the past few years, this situation has changed and intense research has been done in the area of olfaction, which resulted in the 2004 Nobel Prize in Physiology/Medicine, awarded to Richard Axel (Howard Hughes Medical Institute, NY, USA) and Linda Buck (Fred Hutchinson Cancer Research Center, WA, USA).

From the physiological point of view, the senses of smell and taste are chemical senses able to recognize about 10,000 different odours (2004a). There are 1000 different genes (3% of our total genes) that codify an equivalent number of olfactory receptors (proteins), located on olfactory cells in the upper part of the nasal epithelium, occupying an area of 4 cm^2 (EN13725, 2003).

The olfactory tissue is linked to the pituitary gland, related with sexual activity and reproduction, and the limbic system of the brain, part of our unconscious mind and associated to emotions, sexual activity and feelings of pleasure. The odours have so the power of affecting us at a subconscious level.

When the receptors are activated by odorants, the olfactory cells send electrical signals to the olfactory bulb in the brain by thin nerve processes and from here redirected to different parts of the brain. The information from several receptors is combined, creating a pattern that is recognized as the smell of roses for example and memorized (Nobel-prize.org, 2004). The olfactory mechanism is illustrated in Figure 2.9.

In order to flavours and fragrances be perceived, they must be volatile, so the functional groups, molecular structure and molecular mass are important factors. Molar masses of 200 g/mol are common, and rarely overpass 300 g/mol. Besides volatility, odour intensity is also fundamental to impart the typical note of a perfume. Therefore during evaporation, the odour of a perfume composition changes during time due to differences in volatility and odour intensity of its components.

Floral notes are the most appreciated in perfumery and their complexity is a way to allow the memorization of their odour (Calkin and Jellinek, 1994).

How the sensory information is translated and interpreted by the brain has not been disclosed yet. There are two main theories that try to explain how olfactory sense works:

Odorant receptors and the organization of the olfactory system

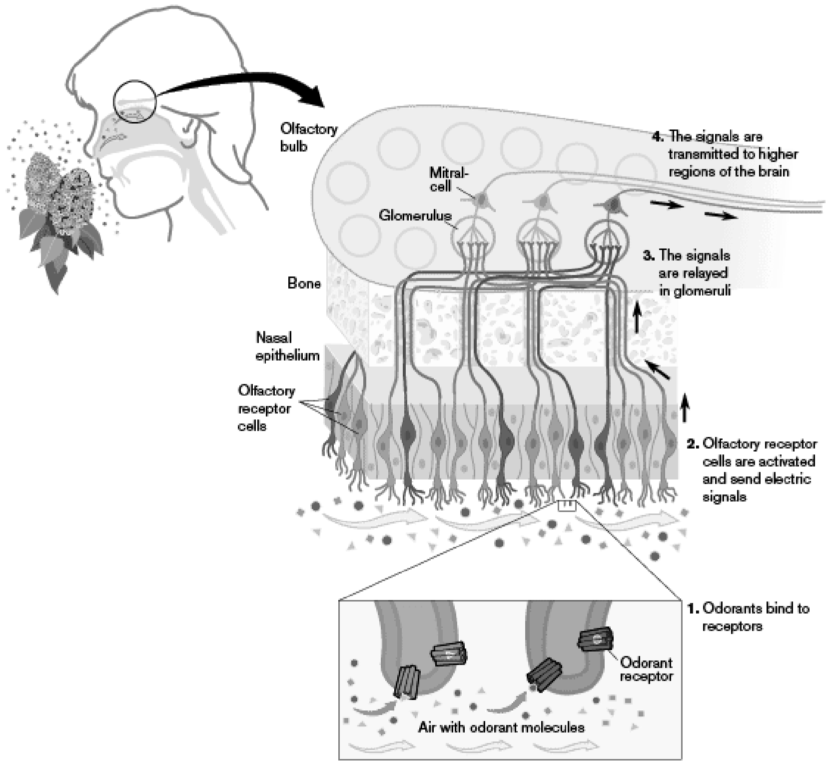


Figure 2.9 The olfaction mechanism (Nobel-prize.org, 2004).

- The shape theory that states that is the shape of the molecules determines the sensory response, so that the molecule must fit into one or more nasal receptors to trigger the smell sensation and its recognition (Amoore, 1970; Beets, 1968).
- The vibrational theory where is admitted that the molecular vibrations are detected by nasal receptors and then translated into odours in our brain (Dyson, 1938; Turin, 1996; Wright, 1977).

Nevertheless both theories have inconsistencies: the shape theory does not explain how similar odours may result from completely different molecules, as is the case of the many different structures of musk molecules, and how the instant detection of many new smells is possible. On the other hand, the vibrational theory does not explain how enantiomers have the same vibrations but smell differently, like the carvone isomers (isomer S smells to caraway and R isomer to mint). Besides it implies that our nose works as a living spectroscope, which is difficult to accept, even though Luca Turin was able to produce new smell molecules based on vibrational theory (in Flexitral, USA).

Therefore, understanding the olfaction mechanism is still a challenge and when the mechanism of smell is found, a wide range of possibilities will be available, namely the development of artificial noses, virtual smells and new delivery systems, and the process of creation of new aroma chemicals with new smells will be easier and less expensive.

2.4 Classification of perfumery materials

The raw materials employed in perfumery have natural origin (animal or plant) or synthetic origin (natural-identical or new artificial fragrant molecules called aroma chemicals). They can also be compounded mixtures to reproduce the scent of a flower or of the ocean, for example. Altogether, the perfumer has to choose between more than 500 natural materials and more than 5000 aroma chemicals (Groom, 1997). In practice only a few hundred are currently used in large amounts (Short, 2002).

The majority of components used in fragrances were natural products until the XIX century. The industrial age introduced synthetic and derived materials, providing perfumers with a wider range of ingredients that are cheaper and have a more consistent aroma. Moreover, the production of synthetics are essential to comply with the huge demand of fragrance and flavour materials for the worldwide market, where the supply based just on naturals would be clearly insufficient (Groom, 1997). Therefore synthetics and naturals are used together, where the natural materials have become gradually into modifying notes that enrich the composition.

In summary, one perfume can be made with three kinds of materials (Perfumers-World, 2004):

- aroma chemicals synthesized from crude oil or isolated from essential oils, making up to 70 to 100% of the perfume concentrate;
- natural essential oils and plant extracts- constituting up to 30% of perfume composition;
- animal products used in very small amounts up to 0.1% in the formulation.

2.4.1 The chemistry of flavour and fragrance materials

Flavour and fragrance components are organic molecules of low molar mass (M<300 g/mol) that have a vapour pressure at atmospheric pressure and room temperature high enough to be partially in the gas phase in order to be perceived by the olfactory sense. The typical odour of a flower or food can be due to a single component or to a group of components, some of them existing only in trace amounts.

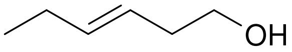
F&F molecules can belong to one of the following categories: aliphatics, benzenoids and terpenoids (Schueller and Romanowski, 1996). Aliphatics are straight-chain compounds, benzenoids contain benzene rings and terpenoids are constituted by a 5-carbon "building block" known as isoprene. These compounds usually have functional groups in their structure, such as alcohol, ketone, aldehyde or ester groups. Most of them are cyclic or heterocyclic. According to the number of carbons in the molecule, terpenoids are classified as monoterpenes (C10), sesquiterpenes (C15) and diterpenes (C20) with 10, 15 or 20 carbon atoms, respectively (Bauer *et al.*, 1997). Some examples of structure types of F&F components are shown in Figure 2.10.

From the point of view of perfumery use, unsaturated alcohols are the most important and impart characteristic notes to compositions (geraniol–rose; linalool–floral). Aldehydes are powerful odour materials used for fruity and citrus notes (citral–lemon; benzaldehyde–bitter almonds). Esters and lactones are related to sweet notes with a characteristic fruity or floral odour (benzyl acetate–banana/ jasmine). Phenols have a powerful odour, besides having antioxidant and bactericide properties, being more active than alcohols (eugenol-clove; 2-phenylethanol-rose). Heterocyclics have a powerful and long-lasting odour (tonalid- musk; vetiveryl acetate - woody).

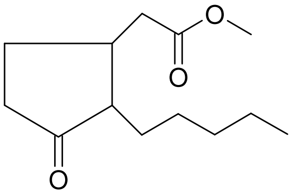
2.4.2 Aroma chemicals

The synthesised fragrant chemicals that are nature-identical products reproduce the existing plant molecule that is not possible to obtain from the natural source, to produce the needed quantity or at reasonable costs and/or to guarantee a constant olfactory quality. In fact, the big companies support some programmes of fragrance-hunting expeditions, like ScentTreck® (Givaudan), to explore rainforests and other exotic environments in order to discover and capture new smells and afterwards

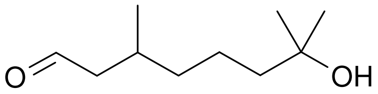
ALIPHATIC FRAGRANCE CHEMICALS



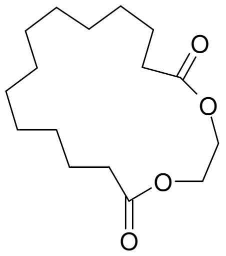
cis-3-hexenol
(cut grass)



Methyl dihydrojasmonate
(jasmine)

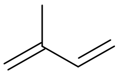


hydroxycitronellal
(muguet)

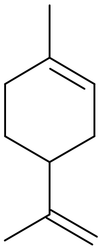


Musk T
(musk)

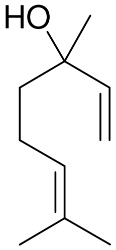
TERPENOID FRAGRANCE CHEMICALS



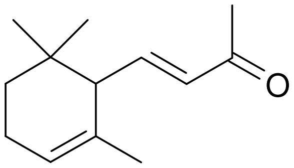
isoprene unit



limonene
(lemon)

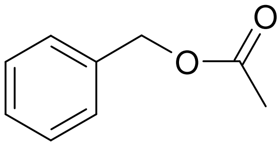


linalool
(fresh floral)

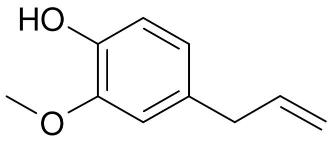


α-ionone
(violet)

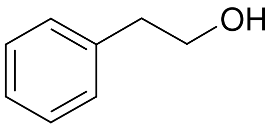
BENZENOID FRAGRANCE CHEMICALS



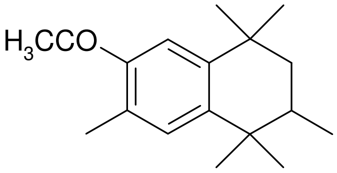
benzyl acetate
(jasmine, gardenia)



Eugenol
(clove)



2-phenyl ethanol
(rose)



tonalid
(musk)

Figure 2.10 Some examples of chemical structures of flavour & fragrance materials.

Figure 2.11 Capturing new scents by portable headspace analysis (source: Newman, 1998).

reconstituting their scents in laboratory (Figure 2.11). The “Living Flower”® research and Hydroponics® (IFF) is another way of screening for new fragrances, by capturing and reproducing the actual scents of the living flowers.

On the other hand, the artificial fragrant materials are new molecules that are not found in nature but that have new and important olfactory qualities, such as the woody amber Iso E Super® and marine helional® (IFF), the jasmine hedione® (Firmenich) and the muguet lilial® (Givaudan) employed in many perfumes.

New molecules are created based on odour-structure models, using sophisticated molecular modelling software. For example, Givaudan researchers start working with a known fragrant compound, creating a 3-D model of its structure, which will be used as a reference. Then, they create new molecules and try to match the structure to that of the reference model. When such a compound is found, it is then synthesized and evaluated (Rouhi, 2003b). Another method followed by Flexitral (Turin, 2002) uses the vibrational theory to create new odorants. The trend is to invent more intensive and long-lasting ingredients, which would be employed in smaller quantities. This would therefore require a low volume production that would have less environmental and health impact and registration would also be simpler (Rouhi, 2003b).

The process of synthesis yields impure components that must be purified usually by vacuum distillation and fractionation. The aroma chemical should comply the specifications of odour quality, chemical composition, cost, environmental and health regulations (Curtis and Williams, 1994). These parameters are strictly enforced, being the perfume industry known as the safest manufacturers and the suppliers of products of fine quality (Curtis and Williams, 1994).

2.4.3 Natural materials

The use of natural products is preferred rather than synthetics in pharmacy, cosmetics and food, according to European and FAO recommendations.

The natural ingredients include essential oils, floral oils, plant extracts, animal extracts from their secretions and isolated natural molecules. Different parts of the plant can be used, namely blossoms, leaves, bark, twigs, buds, fruits, peels, roots, seeds, needles, resins and wood. Raw materials of animal origin are more rare and restrictive, therefore more expensive, like ambergris (whale sperm), musk (Asian deer secretion), civet (civet cat secretion), castoreum (beaver secretion). Also some plants produce odours similar to animal notes, namely costus (sebacious glands of the hair), cumin (sweat), ambrette seed and angelica (musk), cassis bourgeons (male cat). These materials obtained from plants may be chemically very different from animal materials, however their odour and effect are alike. Labdanum and clary sage as sources of amber notes are an example of this (Calkin and Jellinek, 1994). Obtaining animal notes from plants constitutes a very interesting alternative source of these notes since most of the animals live in the wild and are protected species.

The fragrance of a flower, spice or fruit is due to the volatile compounds that cause a pleasant olfactory sensation. The fragrant compounds altogether constitute the essential oil or extract according to the isolation method employed.

Nowadays there is a favourable trend towards the use natural materials for several reasons, namely (Bauer *et al.*, 1997):

- natural products are frequently too complex to be reproduced synthetically or have a considerably superior olfactory quality;
- the synthesis of some organoleptic substances is not economically viable or competitive;

- increasing demand for natural products, mainly due to the psychological effect on consumers that natural is better;
- restrictions on the use of some synthetic materials or compulsory use of naturals.

On the other hand, the quality of natural products depends significantly on several factors, such as geographical origin, harvest time, climate, type of soil and species used.

The worldwide large scale of flavour and fragrance industry led to the cultivation of certain plants as peppermint and lavandin on a very large scale to supply the quantities demanded. Its economic importance have reflected on the agriculture sector in order to develop new varieties to improve yield, oil quality and resistance to diseases and pests (Bauer *et al.*, 1997). Usually the materials produced from intensive cultivation are relatively inexpensive, except for the cases where the production is very labour intensive and has very low yields like rose oil, jasmine absolute, tuberose absolute, orris root oil and orange flower oil and absolute, which prices are extremely high (Bauer *et al.*, 1997).

Essential Oils

Essential oils are the most used natural raw materials in F&F industry, consisting of completely volatile material obtained through distillation. They represent a worldwide market of 700-880 million USD per year and the estimated annual production is 45000 tons (Bauer *et al.*, 1997).

The essential oils can be extracted from different parts of the plant, having several applications in foods and drinks flavouring, fine perfumery, cosmetics, soaps and functional products (such as cleansers, detergents, and fresheners). They are also used in aromatherapy and popular medicine, with considerable benefits since they contain the ‘plant life force’. As essential oils are highly concentrated perfume materials, they should be used in very small amounts.

In the plant material, the essential oil is stored in specific cell structures, namely superficial glandular hairs, oil tubes in the pericarp or oil cells in the plant tissues (Sandra and Bicchi, 1987). The duration and method used to obtain the essential oil will depend on the location of the oil-containing structures and on the difficulty to break the cell walls and free the oil, i.e., the oil in glandular hairs is much more easily liberated than the oil enclosed in roots or seeds, where the oil has to reach the surface by diffusion and then be recovered (Sandra and Bicchi, 1987).

As most natural products, essential oils consist of complex mixtures of hundreds of constituents, with a small group of major constituents that make up the bulk of the oil (composition >1%) and a large amount of minor and trace components. The major components usually determine the overall odour character of the essential oil, while the minor and trace components act as modifiers except when they are intensely odorous and then contribute greatly to the overall scent.

The contribution of the constituents to the odour may happen at three levels (Curtis and Williams, 1994):

- odour quality: the component have an odour that contributes to the essential oil fragrance;
- odour strength, or intensity: the component causes strong impact on smelling;
- persistence or lasting-power: the volatility of the oil depends on the presence of these constituents.

The odour of the essential oil varies during evaporation because the composition is gradually changing as the constituents evaporate since they have different volatilities and odours. The composition of essential oils consists mainly of hydrocarbons and monofunctional compounds derived from mono and sesquiterpenes, phenylpropanoids, amino acids and fatty acids. Essential oils are lipophilic substances, practically insoluble in water, but as they are volatile do not leave fatty stains on paper or cloths unlike other plant fatty oils obtained from soybean or sunflower (Bauer *et al.*, 1997).

Usually, fresh essential oils are colourless or light-coloured yet become darker over time due to oxidation by air. They should also be protected from light and heat that can cause deterioration. Storage should therefore be in a dark, cold, dry place, in tightly stoppered, full, amber glass flasks to minimise these effects.

Essential oils present common physical characteristics: boiling point range from 160 ºC to 240 ºC; densities between 0.759 and 1.096; high refractive indexes; most of them are optically active and so specific rotation is a valuable diagnostic property (Butterfly, 2004).

Extracts

There are four main types of plant extracts, according to the procedure used to obtain them (Bauer *et al.*, 1997):

- *concretes*: result from the extraction of fresh plant material with volatile organic solvents; they contain both volatile and non-volatile components and present low solubility in alcohol, so usually they are not used directly in perfumery; yields are in the order of 0.3% of plant material weight;
- *absolutes*: obtained from concretes by an alcoholic extraction, being used as fine ingredients for perfumes; yields are in the order of 50% of the concrete weight; some absolutes are coloured;
- *resinoids*: prepared by extracting plant exudates (resins, balsams, gums) with organic solvents, giving rise to highly viscous material, mainly consisting of non-volatile and resinous matter; usually are diluted in phtalates or benzyl benzoate to make it easier to work with; they are used as fixatives and are distinct from oleoresins (spices concentrates); the yields are in the order of 50 to 95% of the exudates weight;
- *tinctures or infusions*: are alcoholic solutions obtained by using ethanol or water/ethanol mixtures to extract the odoriferous material; this process was employed for obtaining animal materials but today is seldom used.

Naturals by biotechnological processes

The development of biotechnology has enabled the production of bio flavours and fragrances by fermentation, considered naturals, being one way to meet the world demand for naturals (Rouhi, 2003b). If the substrate is natural, these products are considered naturals since their production result from natural synthesis. In fact, IFF produces natural styrallyl acetate, an ester with gardenia scent, this way: fermentation of acetophenone to styrallyl alcohols, and then these are reacted with natural acetic acid to give styrallyl acetate, using natural processing conditions, meaning that the conditions are similar to cooking (Rouhi, 2003b). Other example is the production of natural vanillin by Rhodia, with a price less than half of the natural extract (Rouhi, 2003b).

Biotechnology has also an important role in the creation of new and more efficient plant species, in order to the plants be better adapted to the field where they grow, more resistant to diseases and produce essential oils in higher yields and with better quality (Petrzilka and Ehret, 1994). For example in Réunion Island the Geranium species are being improved to resolve some phytosanitary problems.

2.5 Production of aromatic plants in Portugal

Portugal is one of the biggest world producers of eucalyptus essential oil, being the only essential oil produced and exported. However, there is a high potential for our country to produce other essential oils since Portugal has "the highest diversity of substrates in Europe; we have all the European climate influences: euro-siberian, continental, atlantic, mediterranean, even macaronesian; we have, together with other Mediterranean regions, 80% of endemic species in Europe; 51% of European biodiversity is concentrated in Iberian Peninsula", according to Professor Eugénio Sequeira in Grande Reportagem (Sousa, 2003). As a matter of fact, Spain having similar characteristics, have already taken advantage of their natural resources producing several essential oils besides eucalyptus oil, namely cistus/labdanum, lavender, lavandin, citrus, rosemary, clary sage, neroli, thymus, basil, marjoram. For example, Biolandes has a large production site in Andalucia that processes 1000 tons of cistus plant per year (Biolandes, 2004).

The overall agricultural production in Portugal is concentrated in a few farms with intensive production, where 2/3 of the agricultural product is obtained just from 20% of the territory. Although the increasing desertification of the interior regions, less than 3.7% of the agricultural soil is effectively abandoned (Sousa, 2003).

There are several plants that are well adapted to Portugal climate and soil and have good organoleptic characteristics. However the cultivation of aromatic and medicinal plants in Portugal is very small, occupying a total of 67 ha (INE data, 1999), mainly concentrated in the South of the country, as can be seen in Figure 2.12. The higher number of exploitations in the South is due to the type of agriculture that is practiced – extensive – more easily adaptable to these types of crops and biological practices.

The idea of cultivation of aromatic plants in Portugal is not new. In the 80's, LNETI has carried out a I&D project on aromatic plants that has pointed out a list of plants with viability to be grown here, such as : *Rosmarinus office. L.* (rosemary), *Cistus ladanifer L.* (rockrose), *Pelargonium roseum l'Her.*

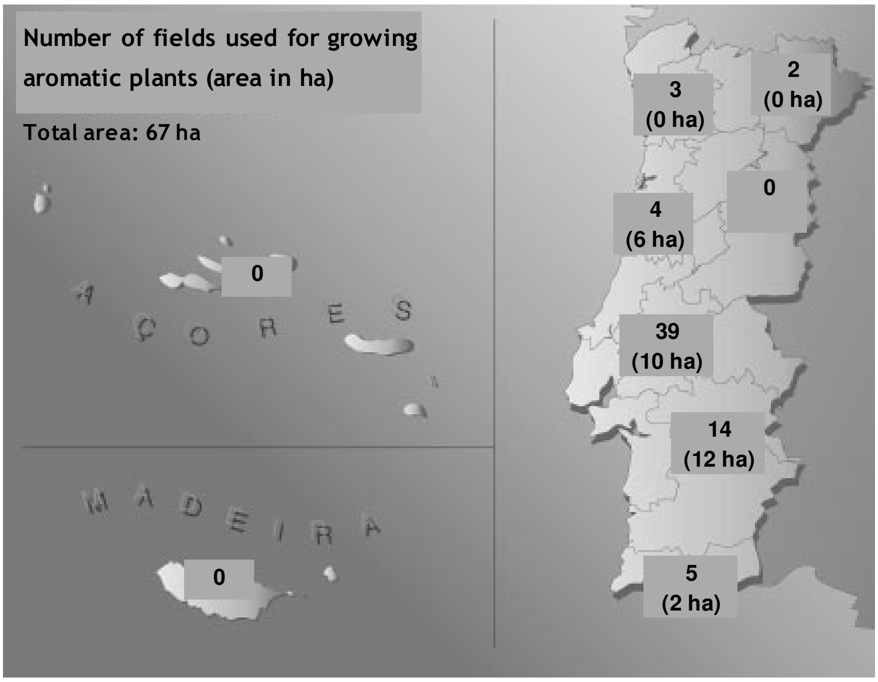


Figure 2.12 Distribution of fields used for growing aromatic plants (source: INE, 1999).

(rose geranium), *Thymus mastichina L.* (thyme "bela-luz"), *Mentha citrate Ehr.* (citrate mint) (Carmo and Frazão, 1987). In particular, rose geranium and thyme "bela-luz" have good olfactive qualities, and citrate mint may be competitive in Europe against American mint. The production estimates of some viable aromatic plants are presented in Figure 2.13.

Recently there are some projects to study and encourage the cultivation and commercialisation of aromatic and medicinal plants, eventually with the use of biological practices, as is the cases of the project "Herbarom" (2003) for the region Entre Douro e Minho, involving the University of Minho, and the project "Plantar Aromas" in Mortágua (2004b).

The selection of aromatic plants for growing is based on spontaneous species or on plants easily adaptable, enabling the exploitation of poor soils without employing any special methods or treatments since these plants are naturally more resistant, little demanding in nutrients and maintenance. The harvest is mainly done manually, although it is being increasingly mechanized.

The cultivation of aromatic plants should be specialised and concentrated in geographical areas, which

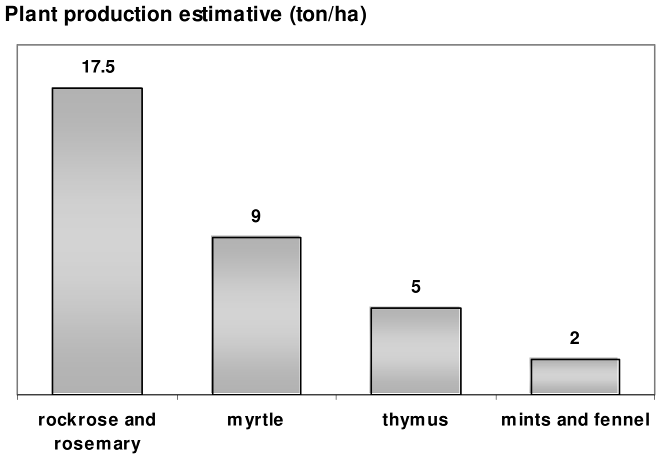


Figure 2.13 Estimative of production per hectare for some aromatic plants with viability of being cultivated in Portugal (source: INETI, 1982).

may include cooperative associations, using plants obtained by micropropagation or vegetative multiplication, in order to: reduce fluctuations of quality (composition); prevent the over-gathering and destruction of autochthon species; constitute an alternative crop for areas with poor soil or climate, and therefore having a positive impact in the local economy and employment; enable the specialisation of the farmer; produce according to demand or through contracts; and establish norms for production and commercialisation, which in turn generate applied research and technological training (Picón and Picón, 1987).

2.6 Production methods of natural perfumery materials

There are three main methods used to obtain the natural raw materials (Bauer *et al.*, 1997): (1) distillation with water or steam; (2) expression or other mechanical processes; and (3) solvent extraction, including supercritical fluid extraction. Figure 2.14 shows schematically the main industrial processes and the natural materials obtained.

A more detailed description of the production methods of natural materials is presented next.

INDUSTRIAL METHODS FOR PRODUCTION OF NATURAL INGREDIENTS

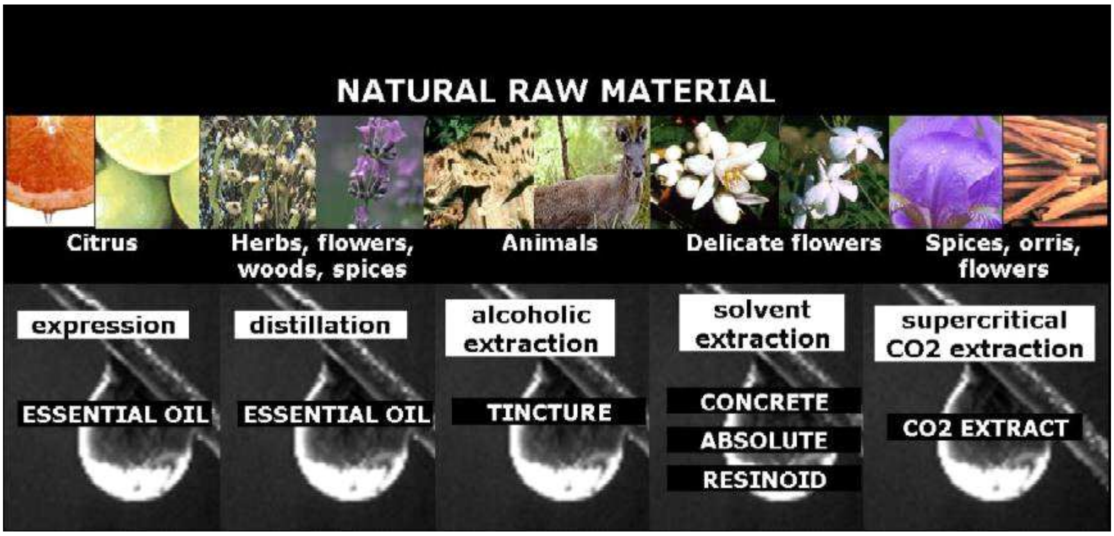


Figure 2.14 Industrial methods used to produce natural materials.

2.6.1 Distillation

Essential oils are mainly obtained from plants by distillation (Figure 2.15). This method is based on the principle that essential oils being volatile are carried along when the plant is in contact with boiling water or water vapour. The essential oil is afterwards recovered by cooling the vapour in a condenser and then a two-phase system is formed, where water and essential oil separate based on differing densities and so the essential oil can be isolated and collected. The formation of smoky notes may contaminate and spoil the essential oil and therefore must be prevented by the careful vigilance of the process of distillation (Curtis and Williams, 1994). Distilled Water or Floral Water is obtained when certain aromatic constituents are water soluble even in trace amounts. Examples of these are rose, lavender, rosemary and sandal waters.

There are three main distillation methods:

- dry distillation, occurs at high temperatures by applying heat directly to the surface of the vessel and is used for oils of high boiling point such as from woods; usually have smoky notes due to the pyrolysis of the plant material;
- hydrodistillation, where the plant material is immersed in the boiling water; sometimes, the distillation is carried out at low pressure in order to have lower temperature and so prevent degradation of thermolabile compounds;
- steam distillation, where a steam flow under pressure (from an outside source) goes through the plant material or the plant material is placed in a basket above the boiling water surface; it is much faster than water distillation so it is most useful for thermolabile and hydrolysable compounds (Curtis and Williams, 1994).

The distilled essential oils have usually unpleasant off-notes, "still notes", especially when using hydrodistillation due to the sulphur-containing molecules resultant from the decomposition of

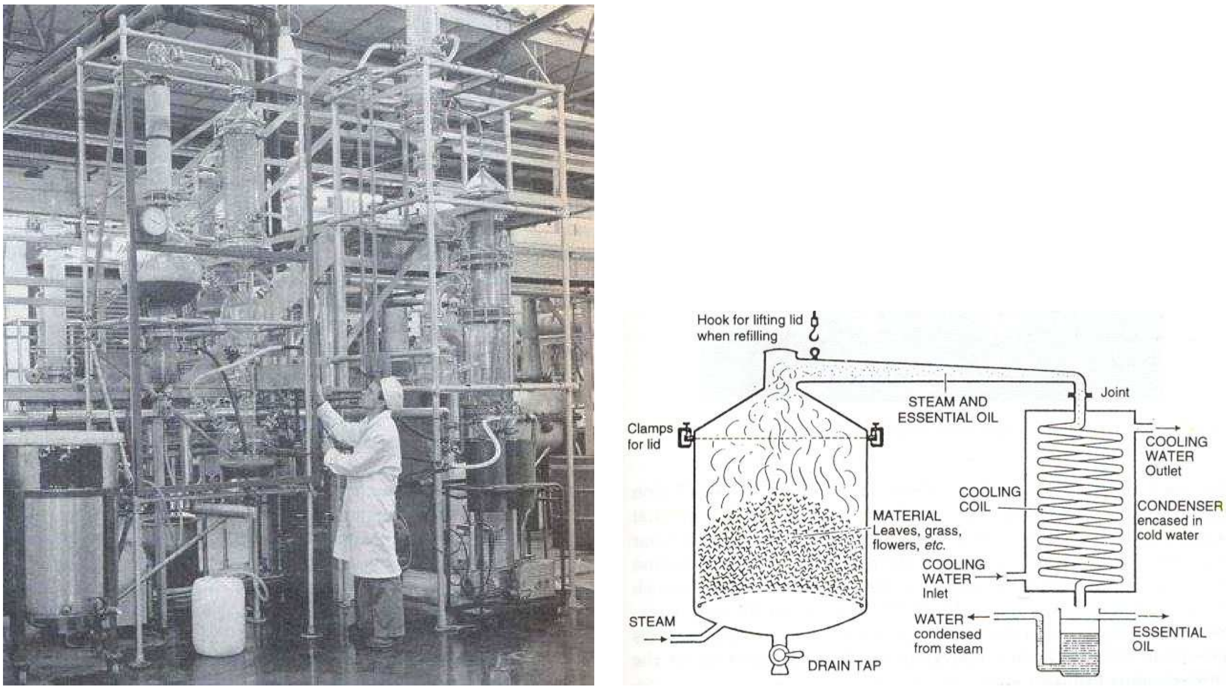


Figure 2.15 Distillation process for producing essential oils.

proteinaceous matter that occurs when boiling green vegetables. Aeration of the oil for several hours or briefly blowing air through it easily eliminates these notes, through evaporation or oxidation of those components to odourless molecules (Curtis and Williams, 1994). However, the smoky notes resulting from a poor distillation can not be removed by aeration, rendering sometimes the essential oil unsuitable for perfumery (Curtis and Williams, 1994).

The oil composition may change during distillation, being the oxygenated compounds extracted first than the lower boiling hydrocarbons because the oxygenated compounds are more soluble in water and so diffuse easier through the cell membranes – hydrodiffusion- and evaporate when reaching the surface. The hydrocarbons have more affinity with the fat of plant material and so they are more retained. Therefore, it is the water solubility that determines the extraction yield rather than the range of boiling points (Sandra and Bicchi, 1987). The distillation is discontinued when the yield increase does not compensate the resources required. Moreover there are reactions induced by temperature that combined with acid pH of 4 to 7, lead to hydrolysis, change of colour, conversion to other compounds and crystallization of high-melting compounds in the condenser (Sandra and Bicchi, 1987).

2.6.2 Expression

The method of extraction of essential oils through application of mechanical pressure or compression is known as expression (Figure 2.16), being the most simple and direct way of obtaining essential oils.

This technique is used to extract citrus essential oils present in the fruit rinds except for lime oil that is sometimes distilled (Bauer *et al.*, 1997). The process consist in crushing the rinds between rollers, the essential oil is released by the mechanical compression and separated by centrifugation (Groom, 1997). The fruits used are bergamot, grapefruit, lime, lemon, citron, mandarin, orange and tangerine.

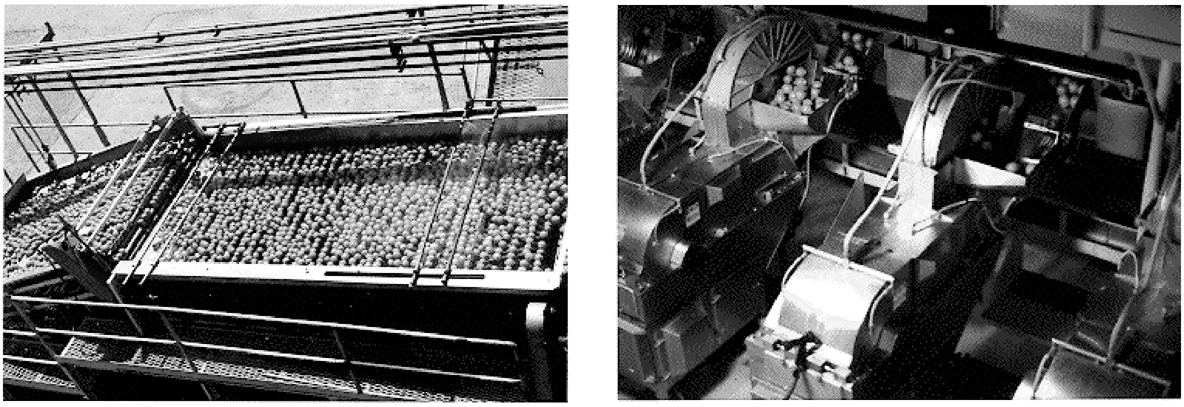


Figure 2.16 Expression process to produce citrus essential oils.

2.6.3 Solvent Extraction

Extraction by volatile solvents or simply extraction is applied in modern perfumery to obtain very delicate materials and fixatives, namely essences from jasmine, cassie, ylang-ylang, clary sage, champac, lily, violet, geranium, mimosa, orange flower, rose, tuberose, lavender and labdanum (Groom, 1997).

The process consists in placing the aromatic plant material on perforated plates and passing the organic solvent through the plant bed, usually hexane petroleum ether, acetone and ethyl acetate (Figure 2.17). All the materials soluble are extracted, including the high and low volatile components, pigments and aromatic waxes. Ethanol is not often used as an extraction solvent due to the high proportion of water co-extracted (Pybus and Sell, 1999) which is not desirable in behalf of the preservation of the extracts. The solution is then decanted and filtrated to remove water and insoluble matter (impurities) and plant material. The solvent is recovered by low-pressure distillation and re-used in the process. The resulting concentrated extract is the concrete, a semi-solid paste.

So, the concretes are free of water since there were used only organic solvents (Curtis and Williams, 1994). The concretes are further processed to obtain a finest perfumery material by making an extraction with ethanol, filter the insoluble matter in alcohol, freeze the solution to about –12 to -15 °C

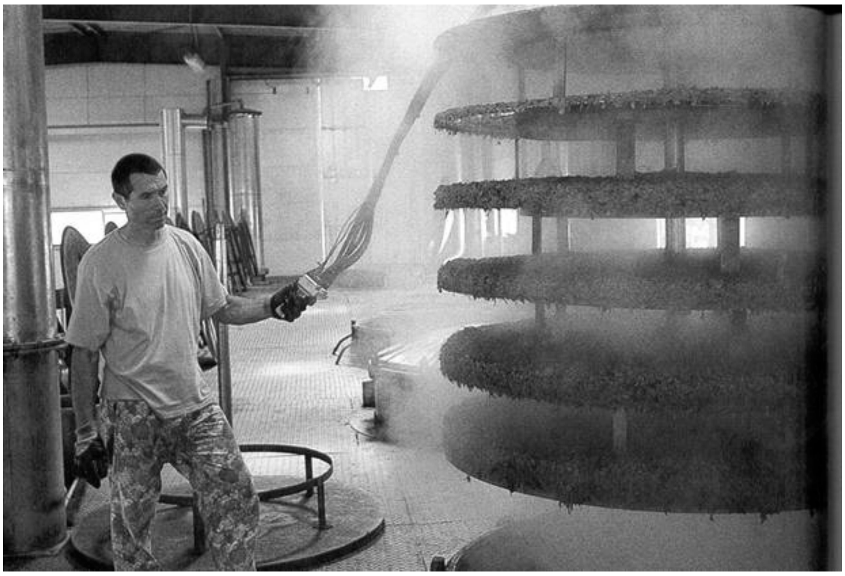


Figure 2.17 Process of extraction with organic solvents to produce natural extracts (source: Newman, 1998).

(PRODAROM, 2004) to precipitate the waxes and finally performing a cold filtration, obtaining the valuable raw material in perfumery, the purest and the most concentrated form– the absolute. The aromatic waxes removed can be used for scented candles, creams and lotions (Groom, 1997).

The required solvent qualities for extraction are: have a low boiling point so that fragrances are not destroyed by heat and leave no residue after evaporating; they also should be more volatile than top notes in order to recover these after solvent evaporation; be selective enough to extract mainly the essential oil and other fragrant materials; non-toxic; non-reactive; available at acceptable purity and price (Curtis and Williams, 1994). The solvent chosen must compromise these qualities in order to obtain a fine extract with good organoleptic quality, affordable and miscible with other perfumery materials.

The main advantage of solvent extraction over distillation is the lower temperature employed, usually below 50 ^{o}C, especially when dealing with thermolabile substances. The extracts usually have a more natural odour since it is richer in the more volatile monoterpenes (Sandra and Bicchi, 1987). This method is also indicated for low yields on essential oils and when in some cases it is desirable to have non-volatile components as fixatives. Therefore, the content of volatile and non-volatile components can be adjusted through the extraction procedure, setting different degrees of fixation and so conditioning the odour development.

The disadvantage is that non-volatile compounds like waxes are also co-extracted. Further processing is usually required, namely distillation or chromatography over silica gel. Also, grinding or cutting the plant material increase the mass transfer area, on the other hand it can also result in evaporation losses due to ventilation of the mills and enzymatic decomposition of non-volatile compounds to volatiles (Sandra and Bicchi, 1987).

Enfleurage is a form of solvent extraction used to produce extracts from delicate flowers, taking advantage of the fact that some flowers continue to produce essential oil for some time after they have been picked. It consists of steeping the flowers in animal fat or non-volatile oil (plant or mineral), which absorb their fragrance and in the end it is recovered by extraction the aromatic fat with alcohol, in a similar way as the treatment of concretes. It is particularly appropriate for the following plants: jasmine, violet, tuberose and jonquil. However, today this process has been discontinued and replaced by organic solvent extraction because it is a very lingering and labour-intensive process, resulting in extremely expensive absolutes.

2.6.4 Supercritical Fluid Extraction

There is an increasing interest in natural products, preferably if they are obtained from plants grown organically and without any additives and contaminants of solvents or pesticides, and obtained in the most sustainable way, using clean technologies such as supercritical fluid extraction (SFE) (Figure 2.18).

SFE extracts have no residues and increased odour naturalness, very close to their original scent. This natural odour character of SFE extracts is becoming much appreciated in food industry and fine perfumery (P.Pellerin, 1991), although they are in some cases more expensive . Nowadays, the major flavour producers in the US and Europe are already supplying SFE extracts of spices, herbs, hops and flowers for food. SFE technology is actually used for industrial production of floral extracts (Danisco, NL), such as jasmine, tuberose, rose, orris and mimosa. In Chapter 4, SFE technology will be discussed in more detail.

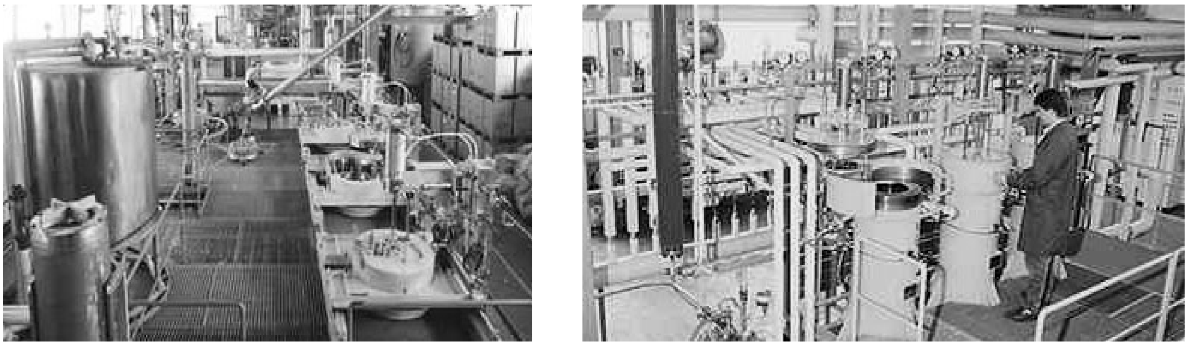


Figure 2.18 Industrial plant for supercritical fluid extraction (Flavex,Germany).

2.7 Adding value to naturals

Frequently there is the need to proceed to further purification, concentration or fractionation of natural raw materials in order to add value to the product or enhance the olfactory properties. The most common cases are: (i) concentration of important key components present in the raw material or (ii) remove unpleasant or unsuitable components from the raw material. The processes used to this end are distillation, partitioning, crystallization and adsorption.

The absolutes are usually coloured due to the presence of chlorophyll or other pigments, which may not be desirable for a perfume composition that is intended to be transparent, for example. The colour can then be removed through adsorption on activated charcoal but there is the risk that odour may change significantly if some fragrant components are also adsorbed on activated charcoal.

Distillation is also applied in the fractionation or purification of natural extracts, in order to render the material more soluble in ethanol (i.e., removal of insoluble monoterpenes), less coloured (i.e., removal of pigments from patchouli oil), more stable or safe (i.e., removal of bergaptene from bergamot oil), while keeping the olfactive quality. Also can be used to produce natural isolates that enhance some olfactory facet (i.e., rhodinol from geranium oil, linalool from rosewood) or are starting materials of high-added value products (i.e., ionones/violet obtained from citral/lemon and iso-eugenol/carnation from eugenol/clove) (PRODAROM, 2004). This is usually performed at very low pressures or vacuum distillation so that the operating temperatures are also low and prevent degradation. Short Path or

Molecular Distillation is a modern industrial technique applied to get natural extracts that are heat sensitive, under high vacuum up to 0.001 mbar.

Although the essential oil and water are only slightly miscible, there is always a small amount of water present in the distilled essential oil. In some cases, it is necessary to remove it for preservation of the oil quality by using drying agents such as anhydrous sodium sulphate. Also some components that are more polar are lost in the distillation water since they are also more soluble in water, such as phenyl ethanol of rose oil. One method to prevent the loss of these water-soluble constituents is cohobation (Curtis and Williams, 1994), which consists of recycling the distillation water saturated with these water soluble components during the distillation process. The essential oil can be recovered by liquid extraction with an immiscible volatile solvent (like hexane), which is then added to the bulk oil after the solvent has been removed.

Rao et al. (2002) proposed the recovery of water-soluble fractions of rose geranium by a solvent extraction with hexane, reporting a recovery up to 8% of total essential oil, rich in organoleptically important oxygenated compounds (88.9-93.9%), comprising the commercial rhodinol fraction (geraniol-citronellol) with a rosy scent and linalool with flowery odour (Rao *et al.*, 2002). Amin et al. (2001) propose an alternative method to recover the water-soluble components by adsorption on polymeric materials, finding that INDION 1014 MN-2 presented greater adsorption capacity (Amin *et al.*, 2001).

Fractionation of oils by adsorption will be explained in more detail in Chapter 5, together with the refinement of citrus oils using this technique.

2.8 Formulation and production of perfumes

A perfume is defined by Calkin and Jellinek (1994) as “a blend of odoriferous materials, which is perceived as having its own unique and aesthetically appropriate identity”. Basically, fine fragrances are constituted by a mixture of fragrant ingredients, the perfume concentrate or blend, and the dissolving media, ethanol.

The creative perfumer or nose before starting to make a new perfume must know how much money can be spent on materials, so that the established maximum price is conveyed (Groom, 1997). If the perfume is directed to mass market at a low retail price, the ingredients must be inexpensive. On the other hand, if the perfumer is asked to compose a fine fragrance, he can use materials of higher quality and thus more expensive. Other factors besides price should also be considered, such as the availability and supply of the raw materials, especially when it will be produced on a large scale, and that the ingredients meet international standards and regulations (Groom, 1997).

The compounding of a perfume mixture relies upon the long experience of the perfumer, which enables him to know how to (Groom, 1997):

- distinguish hundreds of different fragrances by his highly trained sense of smell;
- mix the materials, knowing deeply the effects of materials on each other;
- smooth or sharpen a fragrance;
- bring odours to the same strength level, where their odour is in equilibrium, where no smell is dominating;
- make a perfume to last longer.

The work of a perfumer starts with the request of a new fragrance submitted by a costumer – the brief. In the brief, the client should describe the brand and the product for which the fragrance is being created, giving as much detail as possible and using descriptors to define the kind of fragrance that they have in mind. However, describing a smell is very subjective. For example, the concept of fresh may be associated with water fall, ice, clean air, menthol or green herbaceous. Therefore the perfumer must clarify the idea of the client of the concept of fresh and for that may use images to help the client to express what he wants – concept board. The exchange of information happens during all the period of creation of the perfume, until the final perfume is achieved.

A fragrance evaluator makes the bridge between the creative perfumers and the client, managing the process of fragrance development that includes tasks as: preparing the brief, giving ideas, selecting the odour themes, giving guidelines to the perfumers, analysing test results, preparing the presentation of fragrance to submit to the client, just to mention some. The fragrance itself is evaluated according to performance (quality, strength, substantivity), acceptance (context of product category, branding, market position, target user) and stability (compatibility of product and fragrance) (Ayres, 2004).

The perfume will be finally presented to the consumer with a simple description of its main fragrant aspects, based on a list of few ingredients using the plant names that are more meaningful for people, showing this way the principal fragrant effects and supporting the marketing story of the perfume.

2.8.1 Perfume structure and evaporation

In Perfumery, it is usual to make use of music language to describe odour, being the perfume composed of three main fragrant notes: top, middle and base notes.

Top notes are responsible for the first impression about a perfume, disappearing in a short time, generally some minutes after application. They are composed by the most volatile, light and intense fragrances, with a fresh, green, citric or clean aroma like lemon, lavender or grass scents. They constitute up to 25% of the formula of a perfume (Calkin and Jellinek, 1994). The top notes are also designated as initial, head, peak or outgoing notes.

Middle notes become dominant after the top notes have disappeared, constituting the heart of the perfume and for which it is recognizable. They can persist for more than 4 hours, constituting 30% to 40% of the composition of the perfume (Calkin and Jellinek, 1994). These notes can be described as floral, fruity, spicy or woody-like odour, where the combination of rose and jasmine are present in most of the women's perfumes. They are also known as medium, bouquet or heart notes.

Base notes consist of resinous or crystalline materials that give an underlying and long-lasting odour that gradually fade away and persist for several hours or even days. They work simultaneously as a fixative of the top and middle notes to equalise their volatilities (vapour pressures), to increase tenacity (lasting power on the skin), as well as a blender of the materials. Therefore, base notes constitute the bulk of the perfume, comprising 45% to 65% of the formula (Calkin and Jellinek, 1994). As natural fixatives are used resinous, woody and animal materials, namely labdanum, oakmoss, sandalwood, patchouli, musks, and vanillin. The base notes have other synonyms such as end, low, lower, back, depth, body, dry, fond, dry-out or dry-away notes.

The structure of a perfume based on the combination of top, middle and base notes is known as a classical or triangular structure, represented graphically as the fragrance pyramid (Figure 2.19). These compositions include natural and synthetic ingredients, ranging from simple compositions to very complex mixtures made of hundreds of constituents. The single note fragrance is a simple compound aimed to reproduce the scent of a flower or simple bouquets and simple scents as vanilla or musk, consisting of either natural essential oil of the flower or plant or may be completely synthetic.

Ideally, the perfume should evaporate smoothly from top to base notes, during the 6 to 8 hours that it should last on the skin. The odour of a perfume is changing over time, as it is made of components with different volatilities, according to the profile presented in Figure 2.20: initially only the top notes are perceived; then middle notes start to be noticed, increasing in intensity after some time, while top notes disappear; finally the base notes become noticeable, being the last to evaporate completely. During evaporation, the perfume should keep its overall character or its recognizable accord during all the time. To this happen, the perfume heart should be in the low volatility fraction, which will remain longer on the skin. Occasionally a synergism among fragrant materials occurs, which causes are not known yet, that results in a pleasant odour distinct from the materials separately. Using again music language, it was created an accord.

Recently, a new kind of perfumes appeared, having in a linear or monolithic structure (American style), in which 80% of the formula is a simple composition consisting of four or five components, keeping almost the same scent since the first impression until some hours after application on the skin.

Perfumes are in general extremely sensitive to air, heat and light, so they should be kept in a cool dark place. Nevertheless, the character of perfumes changes over time, loosing firstly the top notes and becoming gradually dark and acidic due to oxidation by air, so that in the end there are some that become strongly unpleasant. Therefore the perfume lifetime should be less than two years after opening the bottle.

One fact to consider when working with perfumes is that the effect of a fragrance may be different from one person to the other due to their specific body chemistry, body temperature and/or emotional condition (Groom, 1997). The perfume can therefore smell and last differently from one person to the other, causing as well different emotions according to the person’s life experience, (since olfactory sensation is intimately related to past memories), personality and fashion. The acceptance of a perfume will always be a highly subjective issue.

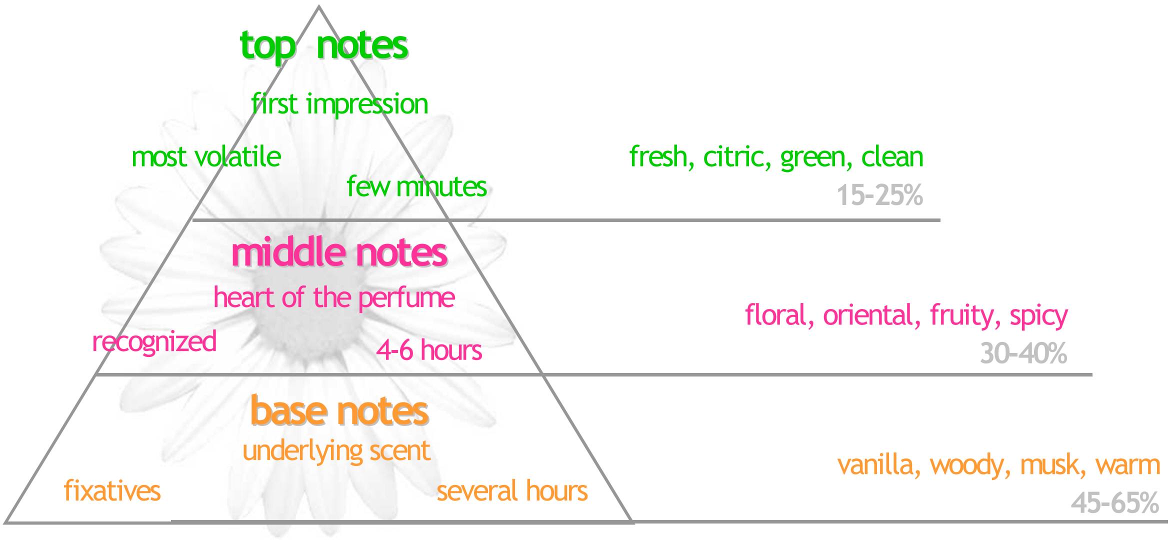


Figure 2.19 Triangular structure of a perfume and relation between top, middle and base notes for a perfume formulation used in the concentration of 12% to 18% in alcohol (Calkin and Jellinek, 1994).

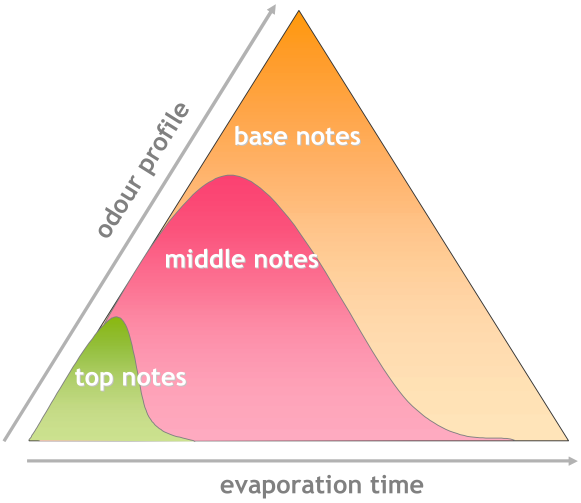


Figure 2.20 Odour profile during evaporation of a perfume with a triangular structure.

2.8.2 Classification of perfumes

There are two kinds of classifications regarding perfumes: (1) according to the concentration of the perfume concentrate and (2) olfactory family.

Fine fragrances can be classified according to the perfume concentration as follows:

- Extrait or Parfum: 15–30% of perfume concentrate in a mixture of ethanol/water 96-99%
- Eau de parfum: 8–15% of perfume concentrate in a mixture of ethanol/water 85-90%
- Eau de toilette: 4–15% of perfume concentrate in a mixture of ethanol/water 80-90%
- Eau de cologne: 3–5% of perfume concentrate in a mixture of ethanol/water 60-80%
- After shave: 2-8% of perfume concentrate in a mixture of ethanol/water 50-70%
- Splash cologne: 2-3% of perfume concentrate in a mixture of ethanol/water 50-70%

Considering the olfactory family, the perfumes are usually based on four main fragrant concepts (Curtis and Williams, 1994; Groom, 1997):

- **floral** : predominant floral note, either as single flower scent like jasmine or rose or combinations of floral notes, also called fantasy notes; these are mainly used in feminine fragrances;
- **oriental** : heavy and long-lasting, exotic, spicy, balsamic character that is reminiscent of Orient; jasmine, rose, lily and tuberose are used as floral notes, while citrus and herbs are used for top notes to give lightness and freshness to the composition;
- **chypre**: non-floral perfumes, based on the accord of oakmoss, labdanum, sandalwood, vetiver, patchouli and civet as base notes; rose, jasmine and carnation as modifying notes; and citrus top notes as bergamot or orange;
- **fougére**: is the main family of men’s fragrances, having the combination coumarin-oakmoss as the fundamental accord and lavender and citrus as top notes, usually together with sandalwood , vetivert, rose and jasmine, with a fresh herbaceous mossy odour.

Poucher (1955) proposed a classification of fragrances within a scale of 1 to 100, based on measurements of evaporation rates (or duration of evaporation). The measurements were carried out by smelling absorbent paper smelling strips at 16 °C, placing the same quantity of fragrance in the same type of paper strip. In the case of solids, it was prepared 10% solution in diethyl phthalate. There were smelled 4 strips per day, every hour. As some fragrances persist for several days, it was set a limit in time. The materials that evaporated in less than one day were given the coefficient 1, while the ones that persisted to the limit time were given the coefficient 100 (Poucher, 1955).

The scale indicates which fragrances are suitable for top, middle and base notes (see Figure 2.21). In this scale, 1 corresponds to the most volatile material – niaouli- and 14 to the slowest evaporating top note – lemongrass, mimosa absolute and palmarosa. The range of middle notes goes from 15 - rose otto, dill and storax oil- to 60 – benzyl phenylacetate, citral and rhodinol formate. Geranium oil is included in the middle notes, with the value 24. Finally, the range 61 to 100 is related to the components with the lowest volatility, most of them used as fixatives, like ambergris, labdanum, oakmoss, sandalwood, vanillin, and so on.

However, some base and middle note fragrances may behave as middle or even top notes according to the quantity used in the composition. Moreover the relative quantity of a fragrance in the composition may influence the evaporation rate of the other components, depending upon the ratio of base notes to middle and top notes fragrances and due to interactions between the constituents or changes that eventually have occurred during maturing time and evaporation.

Carles (1962) established a classification of odorous materials according to their volatility into three groups (Carles, 1962):

- top notes: very volatile products lacking tenacity;
- modifiers: products of intermediate volatility and tenacity;
- base notes: products of low volatility and high tenacity.

The main difference in this classification consists in considering that are the base notes that determine the character and success of the perfume, while the middle notes act as modifiers to mask unpleasant top notes of the base products. The top notes impart a pleasant odour when opening the bottle and potentially influence the consumer decision.

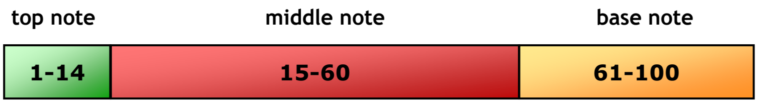


Figure 2.21 Classification of fragrances according to *Poucher (1955).*

More recently there were some empirical approaches by the industry to classify fragrances according to their fragrance concept – floral, oriental, chypre, green, transparent, etc- namely the Rosace of Firmenich (1989), the Fragrance Octagon of Dragoco (1998) and the Fragrance Genealogy of Haarman & Reimer (2001). However, the criteria or methodology used in these classifications is unclear, existing different classifications by different authors.

The classification of odours is useful to the perfumer since the possible valuable fragrances are presented to him in the order of their evaporation and so helping him to work the new perfume, reducing the number of experiments. However this task appears to continue to be very subjective since there are so many different classifications.

2.8.3 Creation of perfume

The creation of a perfume involves a lot of art, science and technology: the art of choosing the right ingredients that concretizes an idea of a perfume with a harmonious and pleasant scent, the science to establish a well-defined structure so that the perfume performs well and the technology that enables the creation, production and characterization of perfumes.

The process of creation is slow (Groom, 1997): the sense of smell gets tired and so it limits the working time per day, it is necessary to let the fragrance blotters to dry before sniffing, testing over lengthy periods to ensure maturation and guarantee that the performance is the one expected in terms of lasting power and odour character after some months in the bottle, at different temperatures, climates and skin type. There is also extensive in-house testing with the staff or outside panels before presenting the final product to the client to ensure that it meets the brief requirements. Therefore, it may take 1 to 3 years to develop a new perfume.

Most perfumes contain 20 to 200 different components (Boeck and Fergen, 1994). In the perfume formula, the perfumer indicates their quantities and strengths, expressed in percentage or parts per mille (1/1000 or ‰). The formula is a highly confidential document and constitutes the know-how of perfume manufacturing (Boeck and Fergen, 1994). The formula safety is protected by passwords, card readers, users ID and the like, as well as by the careful selection of staff, coding the ingredients and formulas and automation of compounding (Boeck and Fergen, 1994).

Perfumes must meet the necessary technical requirements in order to perform satisfactorily, namely (Calkin and Jellinek, 1994):

- sufficiently strong to be perceived;
- diffusive to be perceived by others;
- persistent to last;
- retain its main character to be able to be recognized hours after it has been applied to the skin;
- chemically stable in the final product.

Ethanol with a small percentage of water is the solvent used in fine perfumery. Other solvents like diethyl phthalate (DEP) and dipropylene glycol (DPG) are occasionally used as blenders or synthetic fixatives with low odour. In general, for the final product to get a pleasant scent it is necessary to use only a very small quantity of perfume concentrate.

A fine perfume is based on a relatively simple structure and its uniqueness is imparted by the use of natural products. Poucher (1965) gives the following advice to perfumers: “The key of success is Simplicity, Simplicity and again Simplicity”, meaning that should be used the less number of fragrances as possible in the composition. Nowadays, the consumer appreciates the performance of perfume, concerning its strength and lasting power, besides its pleasant or striking odour.

The perfumer should have an excellent olfactory memory, which can be accomplished by intensive training. Olfactory training is indeed a continuous task for the perfumer (Carles, 1962). On the other hand the perfumer develops his own style and method for creating the fragrances. Two known perfumers have described their creation method, that will be presented next.

Maurer (1958) starts the creation of a perfume with the bouquet (Curtis and Williams, 1994), a floral mixture that is not reminiscent of any known flower, creating a new scent. A mysterious touch is given by adding strong fragrant materials such as sandalwood, vetiver or patchouli. The aroma chemicals can also be employed, imparting an intriguing character to the blend since they are unknown in nature. Speciality bases can also be used to make non-floral perfumes.

Jean Carles (1962) developed a methodology for creating perfumes that performs the trial-and-error experiments in a very systematic way: the composition starts with 2 ingredients that are mixed in a sequence of proportions by weight 1:9, 2:8 and so forth up to 9:1. The mixtures are evaluated on smelling strips and the most harmonious olfactively blend is selected from these 9 solutions. The process is repeated with this solution and a third ingredient, and then successively for the other components. The perfumes have a triangular structure based on the three fragrant notes, starting by creating the accord between the base notes, then adjusting and harmonising the odour by adding the modifiers and in the end add the top notes, in the reverse order of their evaporation sequence. The proportion of fragrance notes is established in order to obtain a balanced evolution of odour during evaporation and to have the right tenacity. This method continues to be the main one used nowadays.

The selection of ingredients should consider the cost, compatibility between the ingredients and with the product base, as well as their odour quality.

Essential oils or other perfume materials blend well if they belong to the same family and the blend between different categories is generally found by experimentation.

2.8.4 Manufacturing of perfumes

The manufacturing of perfume blends – compounding- is now largely automated and computer-controlled, comprising the following stages (Curtis and Williams, 1994; Groom, 1997):

1. the production of the concentrate by mixing the ingredients according to the formula. The formula should contain the list of ingredients in the order in which they are to be mixed, as well as indications on the preparation such as mixing, heating, filtration, and other tasks;
2. maturation period to allow ingredients to mix and stabilize;
3. dilution of the perfume concentrate in ethanol and water to its required concentration according to extrait, eau de parfum, eau de toilette or eau de cologne;
4. maceration in copper or stainless steel containers for a further few weeks;
5. filling, labelling and packing the perfume.

The compounding is most of the times based on weight metering, since it is the most accurate way of measuring the quantities of ingredients (Boeck and Fergen, 1994), not affected by density fluctuations as happens with volume metering. Samples of all batches should be taken for analysis, odour evaluation and reference, as well as to allow traceability of the products delivered.

The quality control in perfume industry is very important because the quality of raw materials influence considerable the smell of the final product. An efficient quality control of raw materials is a way to guarantee the quality and consistency of the fragrance blend supplied to the clients. The quality of a fragrance blend is assessed by the following parameters (Dallimore, 1992):

- Odour: evaluated against a standard by at least 2 experienced evaluators, using smelling strips and smelling immediately after application and after drying down, for a minimum period of 6-8 hours;
- Colour: there are allowed slight variations that happen due to the variability inherent to naturals, however the blend must remain perfectly clear; the turbidity suggests the deficient mixing or the presence of water;
- Density and Refractive Index: give indication on the consistency of the raw materials used;
- Composition: checked by infra-red and gas chromatography analysis, relative to a standard composition for each material;
- Optical rotation: used when are optically active components.

The perfume design and manufacture is a large-scale international business, with a wider range of products being perfumed, especially functional products, to increase the sales. In general, the companies work at some point in the F&F production chain, namely the production of raw materials, production of fragrances or flavours blends and final commercialization of fragranced or food products. There are only few worldwide firms that integrate all this production chain, having their own perfumers, creating and manufacturing the materials and perfumes ready to be sold and even having their own land or exclusive contracts with growers to produce their own natural ingredients.

2.9 The scientific contribution to perfumery

Considering the large quantity of perfume materials and the increasing number of formulations for different applications, it is an overwhelming task to test each perfume composition in the product base and during all the stages of its lifecycle - empirical approach. Alternatively, each perfume material can be tested individually just in a standard formulation and it is assumed that it will have the same behaviour in the final product - this is a semiempirical approach (Calkin and Jellinek, 1994). Both approaches are very subjective, depending on evaluator's judgement, and considerably time consuming and costly.

Therefore, there is the need of a methodology that effectively give some guidance to the perfumer and does not require so extensive testing – a systematic approach that seeks for relationships between the several aspects of performance of fragrances and their physical and psychophysical properties and then try to relate these properties to their chemical structure. The testing is thus focused on key components.

However, perfumers have not been able to predict performance yet due to the complexity of the phenomena involved and the difficulty in measuring the necessary data or finding published data (Calkin and Jellinek, 1994). Therefore many perfumers renounced to this approach.

This situation will tend to change with time and economic pressure on the perfumery industry and the development of technical analysis, more and more sophisticated and sensitive, that enable determination of vapour pressures and concentration of odorants in the headspace with increasing accuracy.

2.9.1 Isolation methods

Fragrance materials are isolated by distillation, extraction and adsorption methods, such as: hydrodistillation (HD) by a Clevenger apparatus and simultaneous distillation/extraction (SDE) using a Likens-Nikerson equipment, extraction with volatile organic solvents (SE) or liquid or supercritical carbon dioxide (SFE), and headspace technology (HS), including solid phase microextraction (SPME) and purge-and-trap (P&T). The selection of the isolation method depends on the nature and amount of fragrant material and plant, and the qualities wanted in the extract (Pybus and Sell, 1999).

Fractional distillation (FD) and preparative high pressure liquid chromatography (P-HPLC) or preparative gas chromatography (P-GC) are used for the fractionation of the extracted materials into individual components or fractions with interesting organoleptic characteristics, to analyse or investigate further.

2.9.2 Analytical methods

The identification and characterization of new materials, in many cases present in trace amounts, requires the use of very sophisticated and expensive technology such as chromatographic and spectroscopic techniques (Bauer *et al.*, 1997).

Gas chromatography (GC) is the most efficient separation process of complex volatile mixtures such as natural materials. It can be associated with flame ionization detector (FID) and mass spectrometer detector (MS) for the quantification and identification of components, respectively. It is therefore widely used in quality control. Selecting the right GC column packing material and temperature programme, the GC/MS method can be optimised to analyse almost any volatile sample.

The retention times in GC columns are constant if the operating conditions are kept constant, and so they are also used to identify components. The Kovats retention indexes (Kóvats, 1958) normalize the retention times, making possible the comparison of data obtained in different equipments and operating conditions, for the same type of capillary column.

GC analyses can give important information on fragrant materials: the retention times obtained in non-polar columns is strongly related with their boiling points, while the retention times in a polar carbowax-type column give a good information on the polarity of the compounds and also an indication of the relative volatilities of the components (through retention times) (Calkin and Jellinek, 1994).

The limitations of GC/MS technique overcome when one is dealing with completely unknown substances and in many other cases the identification is ambiguous. In this case, other complementary analysis must be performed for identification and/or confirmation of identity, such as nuclear magnetic resonance (NMR), infra-red spectroscopy (IR) and ultra-violet spectroscopy (UV). The main drawback of NMR is the requirement of a pure component, which is difficult to obtain from natural products, once many times these components appear only in trace amounts.

Sniffing technology or gas chromatography/olfactometry (GC/O) combines GC separation and sensory analysis, where the nose, as a biological sensor, is still more sensitive than the best detection equipment, namely electronic noses. On the other hand, the sniffing technique requires the use of trained panellists, what makes the measurements somewhat subjective and is extremely difficult to obtain consistent values.

Headspace technology, also known as ‘Living Flower Technology’®, is a non-destructive and portable sampling method that was first developed by IFF to capture the exact composition of a scent. For example, a living flower is encased in a glass vessel, shaped in a way that does not damage the plant. The fragrance released to the surrounding air is drawn by a small vacuum pump for several hours through a glass tube filled with adsorbent material – trap - where the fragrance will be trapped and concentrated while air and water will pass through. There are several trap materials, namely activated charcoal, Tenax or other polymeric matrices, that have different affinities and capacities towards volatiles, depending on their polarity and volatility (Pybus and Sell, 1999). In the end, traps are sealed, kept in a dark and cool place, until they can be analysed.

Later in a laboratory, the fragrance will be recovered from the adsorbent and analysed by GC/FID, GC/MS, GC/Sniffing, NMR and other complementary techniques to identify and quantify the components existing in the headspace, determine the important odorants and their intensity, and finally reconstitute the scent.

Headspace analysis is particularly advantageous in the cases that it is not possible to extract it from the natural source or when the extracts obtained by common techniques are not able to represent the flower’s scent (Pybus and Sell, 1999).

This technique may also be used to produce new natural-identical ingredients, usually cheaper than those from traditional methods.

Electronic noses consist of an array of 4 to 32 chemical sensors that respond to volatile odour compounds in the air. They are employed mainly in wine and food industries to follow fermentation process or assess food quality. The main advantage of using electronic noses instead of people is that those do not loose sensibility nor get tired or saturated. However for flavour and fragrance applications, there is a problem in finding selective and extremely sensitive sensors (for very low concentrations), therefore panellists continue to be used as the best available mean to detect odours. Moreover, the measured signal is influenced by humidity in air, the data handling requires high degree of computation and needs chemical and sensorial calibration of all the sensors by GC-MS and dynamic olfactometry, respectively.

2.9.3 Physical basis

Volatility

Volatility shows the tendency of a certain fragrant material to pass to the vapour phase and so determines the staying power of that fragrance on the substrate (Calkin and Jellinek, 1994). This is the most important property in perfumery, since aromas are only perceived in the vapour phase. In fact, Poucher (1955) and Carles (1962) developed methodologies for perfume composition based upon volatility.

As it was referred above, perfumers have measured volatility by smelling a paper strip with the perfume material, during several periods of time until the odour disappears. Most of the classifications of volatility by perfumers are based on this method (Carles, 1962; Poucher, 1955), although a paper strip is not a good representative material of the usual substrates in perfumery, such as skin, hair, soap or cloth. The vapour pressure at normal ambient temperature (20 ^{o}C) is a more general and independent measurement, but there is little published data (Calkin and Jellinek, 1994), mainly for those materials recently introduced into the market.

Some authors suggest that the best perfume compositions have materials with comparable vapour pressures (Calkin and Jellinek, 1994), stating that if these values are very different, the composition becomes rapidly unbalanced because the most volatiles are lost faster than the others.

Volatility is intimately related to molecular size and functional groups. Therefore, for the same family of compounds, the larger the molecular size the lower is the volatility and higher is the staying power.

The existence of functional groups with oxygen atoms in the molecule gives rise to stronger molecular association, which reduces greatly the volatility. The effect of functional groups on volatility depends on molecular size, number of oxygen groups and type of functional groups. Therefore, smaller molecules and higher number of O atoms, results in lower volatility. When considering the effect of type of functional group, alcohols and acids (hydroxyl functional group –OH) are less volatile than aldehydes, ketones and esters (carbonyl functional group C=O), since the first promote hydrogen bonding that originates the stronger intermolecular forces.

In general, the functional group effect overcomes the molecular size effect. During evaporation, the intermolecular bonds are broken, the molecules escape to the gas phase and become available to be detected by olfaction.

Solubility

The solubility of the fragrant materials in the media is very important, determining the stability of the perfume mixture and its appearance (turbidity appears if occurs immiscibility).

Solubility is closely related to the polarity of components, being known that "like dissolves like". Therefore, acids and phenols, highly polar, dissolve in water and alcohol, whilst ethers, ketones and hydrocarbons of low polarity are soluble in oils and waxes. In multiphase systems like emulsions and creams, there are both organic and aqueous phases and usually the materials are considerably more soluble in one of the phases.

In a blend of fragrant components, as in a perfume or even an essential oil, the components will be more or less retained in the liquid phase according to their affinity towards the solvent and between themselves. If there is high affinity between perfume materials and solvent, the tendency of this material to evaporate is diminished, resulting in low odour intensity. The solvent affects differently the volatility of each ingredient and so distinct odour effects can be obtained for a given blend just by varying the solvent used. Therefore, the base employed has a very important role in a perfume composition since it: (i) may contribute to the odour, (ii) will determine the perfume odour profile by affecting evaporation rates and (iii) may react with fragrant compounds changing their odour (Calkin and Jellinek, 1994).

Polarity

Polarity influences volatility and solubility. It is an important property for fixation and substantivity, and many GC analyses are based on polarity differences or polar affinities. Polarity decreases rapidly with the increase of hydrocarbon chain length, which is considerably non-polar. A mixture of components has an intermediate polarity of the one of its individual components. The organic families are ordered according to their polarity in Figure 2.22.

Odour threshold value and odour value

The odour threshold value or threshold concentration is defined as the lowest concentration at which a chemical compound can be distinguished with certainty from a blank under standard conditions (Bauer *et al.*, 1997). In another words, they correspond to the minimum concentration of a fragrant molecule at which the nose is able to detect it as a smell. Threshold values can be further divided into detection threshold and recognition threshold, making the distinction between the minimum concentration at which a given material is detected but not necessarily recognized and the minimum concentration to be identified by its odour, respectively.

Odour threshold values constitute one of the most valuable data for perfumery, since they condition the minimum amount to use of an ingredient in a formulation so that it will be perceived. They are also important indicators of their performance: comparing substances with similar volatilities and at the

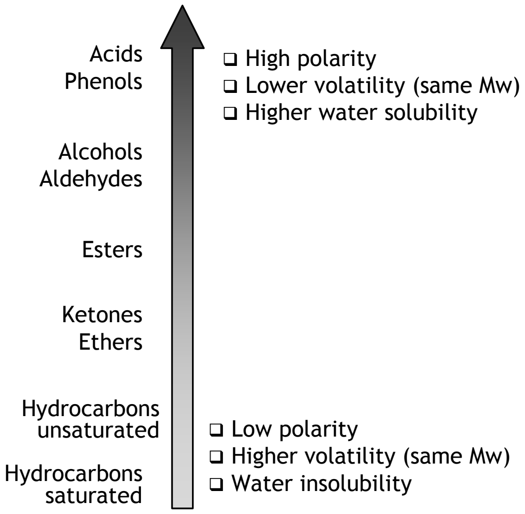


Figure 2.22 Polarity of different kinds of fragrant compounds and the relation with other physical properties (Calkin and Jellinek, 1994).

same concentration level, the lower threshold compounds show a stronger odour. Often it is found that the key components of a flavour or fragrance are only present in trace amounts but have extremely low threshold and so they strongly impact the overall sensory impression.

On the other hand, the olfactory impression varies according to the concentration of the component, with intensity increasing with the increase of concentration. This variation is not linear, following an exponential law, described by Stevens (Stevens, 1957).

Combining the information of odour threshold values and gas phase concentration, a parameter named Odour Value is defined. This variable is used to infer how an aroma chemical can contribute to the overall character of a perfume or flavour and evaluate their impact during the evaporation of the perfume. The OV values are also important to select fragrant components that can "mask" some unpleasant odours, for example, the odour of smoke in a *caffe* or in the cloths, or the smell of some ingredient used in a cosmetic product that has undesirable unpleasant off-notes and its odour must be masked in order to commercialize the product.

Odour performance

The creative perfumer, which is also known as 'Nose', has to comply with economic restrains when designing a fragrance in order to get the best quality at the lowest possible cost. A fragrance is efficacious if it is noticeable and preferably that it has a high performance, i.e., achieves maximum odour effect at the lowest possible concentration. The cost reduction can be achieved by using inexpensive ingredients, lowering the concentration of perfume base and optimizing the composition in terms of the number, quality and quantity of ingredients. Therefore, the use of high performance materials contributes to achieve this goal, since smaller amounts of materials will be employed to produce the same effect.

2.10 Conclusions

The main aspects related with perfumery industry and applications were herein covered, starting from the production of raw materials to the formulation and production of perfumes, as well as the scientific contribution to perfumery in order to make a systematic approach. The prediction of odour values of a perfume mixture can help the perfumer in the compounding task, reducing considerably the trial-and-error procedures.

Considering the scarcity of technical and scientific information in the perfumery area with relatively few publications, the following chapters of this thesis aims to contribute to the study, development and disclosure of methodologies useful for F&F applications.

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CHAPTER 3

CHARACTERISATION OF TWO PORTUGUESE NATURAL MATERIALS

In this work, one of the objectives is to extract essential oils from natural Portuguese resources as potential raw materials to compound perfumes.

The selection of the plants herein studied was based on their olfactory quality and viability of being cultivated in Portugal. Therefore two plants have been chosen as being representative of different fragrant notes that blend well: rose geranium (*Pelargonium sp.*) as a floral middle note and rockrose (*Cistus ladanifer L.*) as an amber base note.

This chapter describes the characterization of the natural materials obtained from geranium (Gomes *et al.*, 2004) and rockrose (Gomes *et al.*, 2005), which resulted in the publication of two papers in *Journal of Essential Oil Research*.

3.1 Introduction

In Portugal, there is a wide variety of aromatic plants that grow in the wild all over the territory, due to the existing diversity of climate and soil, favourable to their growth. In this work, in a first stage, several plant species that could be used in producing natural raw materials for perfumery were screened in the wild, in our home gardens and in public aromatic gardens (Figure 3.1), such as Jardim de aromáticas da Fundação de Serralves (Porto), Jardim Botânico da Ajuda (Lisboa), Jardim Botânico do Porto and Parque Biológico de Gaia (V. N. Gaia). It was also created an aromatic garden in order to make propagation and cultivation of important species for our study (Figure 3.2). The following plants were collected in different places in the country and their essential oil extracted and analysed: Thyme (*Thymus mastichina* or bela-luz), Honeysuckle (*Lonicera sp.*) and Rosmaninho, one kind of lavender (*Lavandula pedunculata*) from Montesinho (mountain region in the North of Portugal); Rockrose (*Cistus ladanifer*) from Alentejo and Beira Interior (Interior Center and South of Portugal); Rosemary (*Rosmarinus officinallis*) from Beira Interior (Interior Center and South of Portugal); and Rose geranium (*Pelargonium graveolens sp.*) from Alentejo (South of Portugal). Afterwards, the plants with economic potential to be grown in large extensions and with important applications in perfumery were selected. Therefore, two plants that fulfil those criteria have been chosen, being representative of different fragrant notes used in many perfumes: rose geranium (*Pelargonium sp.*) as a rose floral middle note and rockrose (*Cistus ladanifer L.*) as an amber base note. In the case of rose geranium, the plant was propagated and small pilot plantings (Figure 3.3) were created to produce the plant necessary for the extraction experiments described in Chapter 4, while the rockrose used in this work was collected in the wild. The characterization of these two essential oils is described next.

Figure 3.1 Aromatic garden in Fundação de Serralves, Porto (Portugal).

Figure 3.2 Aromatic garden created within this work, Vila da Feira (Portugal).

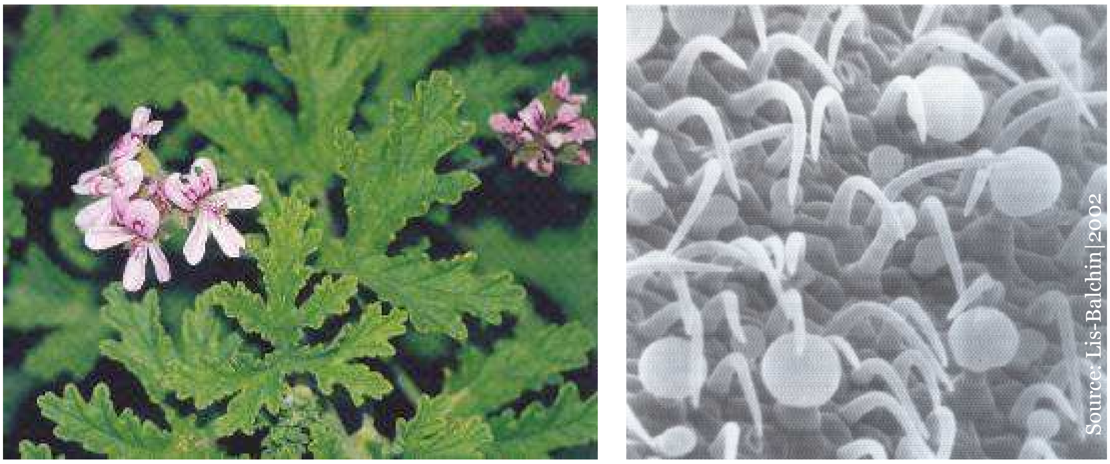
Figure 3.3 Pilot planting of geranium, Oliveira do Douro e Miramar (Portugal).

3.2 Portuguese-grown Geranium-rose (*Pelargonium sp*) essential oil

Geranium oil is widely used as a floral substitute of the rose scent, being therefore one of the most valuable natural materials for the perfumery and cosmetic industries (Bauer *et al.*, 1997). The very recent publications on geranium oil (Babu and Kaul, 2005; Lawrence, 2005) show that it continues to be an interesting natural material.

Geranium oil is obtained by distillation of the green foliage, and sometimes also from the flowers (very fragrant), of *Pelargonium graveolens* or some of its hybrids (Figure 3.4). The essential oil is contained in secretory sacs at the end of glandular hairs (glandular trichomes) that are mainly located on both surfaces of the young leaves and stems, buds and different parts of the flowers.

Though originated from South Africa, it is reported to grow wildly in Réunion Island, Morocco and Algeria (Gulati *et al.*, 1977). This aromatic plant is cultivated extensively in Réunion Island (Bourbon type) and Madagascar, Egypt (North African type) and China, and more recently in India. The Bourbon type presents the best quality and is therefore the most expensive and the most used in the composition of fine perfumes, but its annual production is small. The biggest producer of geranium oil is China, representing 61% of the supply (see Figure 3.5).



Source: Lis-Balchin|2002

Figure 3.4 Rose-geranium plant (Pelargonium graveolens) and the trichomes that contain the essential oil.

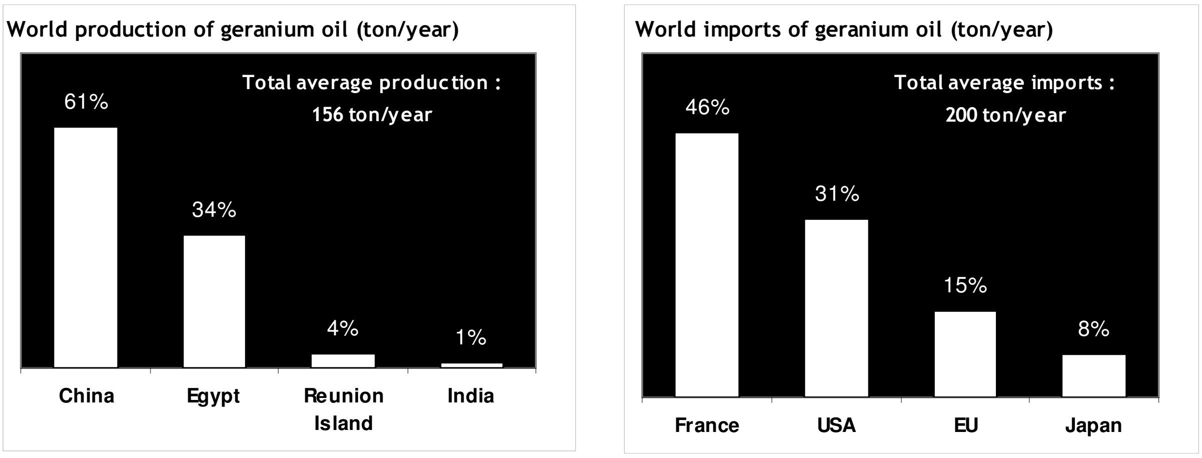


Figure 3.5 Worldwide production and imports of geranium oil (source: Dermarne, 2002).

The major importers of geranium oil are France and USA, followed by the rest of EU and Japan, trading in total 200 ton/year. As the total need is 220 tons (Demarne, 2002), already excluding the trade between USA and EU and within EU, there is a demand higher than the supply of about 20 ton/year, which represent about 3 to 7 million dollars.

Geranium oil is employed as such or as a further refined material, which then demands advanced technical knowledge of process engineering (Gulati *et al.*, 1977). The refinement is accomplished by means of a fractionation process plant to extract fragrant isolates like commercial rhodinol (mixture of linalool, citronellol, geraniol), or to obtain a high-grade essential oil with the removal of light fractions of terpenes, known as terpeneless essential oils.

This essential oil is a natural insecticide and antiseptic. In aromatherapy is most appreciated by its tonic and calming properties (Lawless, 1995). It also has relaxant and potent antimicrobial pharmacological properties (Lis-Balchin, 2002). Moreover, geranium oil presents a great stability, including in alkaline media, which is advantageous for soap applications. The concrete and absolute of geranium have more tenacity than the essential oil but their production is very small.

The norm ISO 4731:1972 defines geranium oil as "The oil obtained by steam distillation of fresh or slightly withered herbaceous parts of *Pelargonium graveolens L'Heriter ex Aiton*, *Pelargonium roseum Willdenow* and other undefined hybrids which have given rise to differing ecotypes in the various geographical areas". This norm also sets the citronellol concentration for geranium oils according to their origin: Bourbon (42-55%), Morocco (35-58%), Egypt (40-58%) and China (40-58%).

The yield and quality of geranium oil vary according to several factors, namely plant variety, climate, soil, cultivation, harvest and method of distillation. The quality variations of the oil is related to the relative concentrations of 8 components (Demarne, 2002): (E) and (Z)-rose oxides, linalool, geraniol, citronellol, isomenthone, guaia-6,9-diene and 10-epi-γ-eudesmol.

Geranium oil, as any natural material, is very complex with hundreds of components, however only 30 components are regularly present in the oil and constitute the bulk composition (about 90%), each of them with a percentage amount higher than 0.3%, and the remaining exist only in trace amounts (Demarne, 2002).

3.2.1 Organoleptic characteristics

Geranium oil has a greenish-yellow colour and a rose-mint odour.

It is interesting to note that the odour of the plant changes according to the time of harvest: from lemon like to rose like when the leaves turn from green to yellow (Gulati *et al.*, 1977), and from minty to rosy during flowering (Lis-Balchin, 2002).

The colour of geranium oil also varies with the origin (Demarne, 2002), from amber-yellow of Egypt's, through yellowish-green or brownish-green tonalities to green of Bourbon's.

The main constituents of the geranium oil are citronellol (sweet rose-like odour) and geraniol (flowery-rose-like odour), which occur in different proportions according to the origin of the oil (Gulati *et al.*, 1977). Both Bourbon and North African types contain unusual high quantities of (-)-citronellol, isomenthone and monoterpene formates. However they can be distinguished by the presence of different constituents such as guaia-6,9-diene in Bourbon oil and 10-epi-γ-eudesmol in the African type. The Chinese oil is similar to Bourbon type, having higher content of citronellol (+40%) and lower content of linalool and geraniol (Bauer *et al.*, 1997; Teisseire, 1987).

Freshly distilled oil has an unpleasant odour due to the presence of dimethyl sulphide that disappears during ageing of essential oil, by promoting aeration or by filtering. The colour also changes with time, turning gradually from green to yellow (The-Good-Scents-Company, 2004).

3.2.2 Perfumery and cosmetic applications

Geranium oil is used in high quality perfumes, having a floral green herbal fresh minty earthy scent and giving body to the perfume since it is long-lasting. It may also be used to compound rose bases and artificial rose oil. Many rose waters, appreciated by women to clean the skin, are based on geranium oil as well. It is also applied in toiletries, where its herbal character imparts a natural concept.

This material is often used as a heart note in masculine perfumes, adding a floral character to green and fougère compositions, as in Kouros by Yves Saint Laurent or Hugo by Hugo Boss (see Figure 3.6 – a). In women's fragrances, geranium constitutes a heart note in green florals and in classical chypres as Chypre Coty (1917), Cabochard, Gres (1958) and Dioressence by Dior (1979).

In modern perfumes it was used in combination with mandarin in Giorgio by Armani (1981) and together with a strong floral heart of mimosa, bergamot, mayflower, hawthorn and juniper in Paris by Yves St. Laurent (1983). As example, it is shown the composition of Paris in Figure 3.6 – b. Geranium oil blends well with artificial musk, vanillin, bergamot oils, patchouli, clove and heliotropin (Wells and Lis-Balchin, 2002).

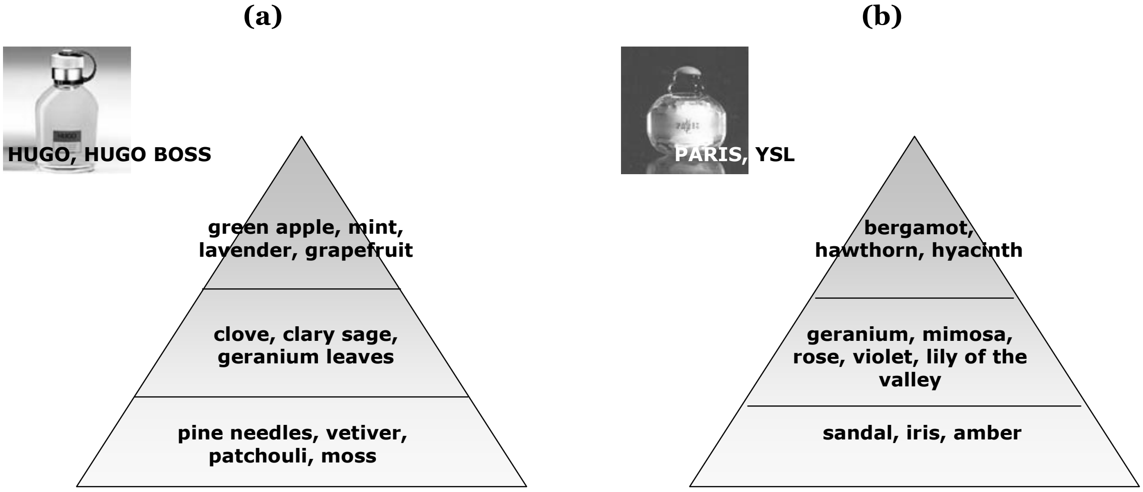


Figure 3.6 Composition of two perfumes with geranium note: (a) men's fragrance, Hugo, Hugo Boss(1995); (b) women's fragrance, Paris, YSL (1983).

3.2.3 Review on geranium characterization

The rose geranium plant grows wildly in the South of Portugal, although it is not native from Portugal. In fact, good olfactory quality geranium oil had already been produced on a small scale in Portugal and exported to England (Seabra and Carmo, 1989).

Several reviews on geranium oil composition studies were published by Lawrence (Lawrence, 1984; 1988; 1992a; b; 1994; 1996; 1999a; 2003).

Guerere et al. (Guerere *et al.*, 1985) compared different extraction methods, namely hydrodistillation both in copper still and in laboratory glass apparatus and solvent extraction with hexane. They found that the linalool contents in the oil were considerable higher than in the concrete, while the geraniol concentration was lower. To justify this fact the authors proposed a reaction mechanism of transformation of geraniol in linalool through water vapor action, which is catalyzed by copper ions Cu^{2+}.

Van der Walt and Dermane (Walt and Demarne, 1988) examined the morphology and oil composition of two close geranium species, *Pelargonium graveolens* and *Pelargonium radens*, and their hybrids. Their composition was very similar except for minor components, such as the phenylethyl tiglate that occurred only in *P. graveolens*, while β-bourbonene and guaia-6,9-diene were found just in *P. radens* and the hybrids contained the three components. The same authors published also a work on the comparison of *Pelargonium* species to find the origin of the cultivar *cv. Rosé* grown in Réunion Island (Demarne and van der Walt, 1989).

Seabra and Carmo (Seabra and Carmo, 1989) presented a study on the cultivation of geranium in Portugal. They found that Portuguese geranium oil was intermediate between Bourbon and African type, containing both 10-epi-γ-eudesmol (African) and guaia-6,9-diene (Bourbon), which imparted its special and appreciated olfactive characteristics. The content in citronellol was higher than commercially available geranium oils, whereas the geraniol and linalool contents were lower.

Machado et al. (Machado *et al.*, 1993) employed headspace gas chromatography to characterize the composition of CO_2 extracts and the oil obtained by steam distillation from a geranium plant also cultivated in Portugal. The authors found that the aroma of the CO2 extract was more closely resembled to the natural aroma and of improved olfactory quality due to the higher content of geraniol and citronellol and lesser amount of linalool.

In the present work, the composition of geranium oil obtained from *Pelargonium hybrid* cultivated in Portugal is reported, revealing different characteristics from those previously published by making use of several isolation methods and plant preparation.

3.2.4 Materials and methods

Plant material

The samples consisted of foliage of rose-geranium plant *Pelargonium hybrid*, using fresh/green (Figure 3.7-a), fresh /yellow (Figure 3.7-b), air-dried/green and oven-dried/green leaves. The samples were collected in October-November, after the flowering season, from plants cultivated in the North of Portugal (humid climate). These plants were propagated by stem cuttings from a wild plant found in the South of Portugal (dry climate). The crops were two years old.

To study the influence of harvest time, there were samples collected in July, during flowering period, consisting of fresh green leaves and flowers.

Oil and extract isolation

The geranium essential oils were obtained by hydrodistillation of plant material (25-30 g) in a Clevenger-type glass apparatus for 2h (Figure 3.8-a-b).

Figure 3.7 Leaves of rose geranium fresh plant : (a) green;(b) yellow.

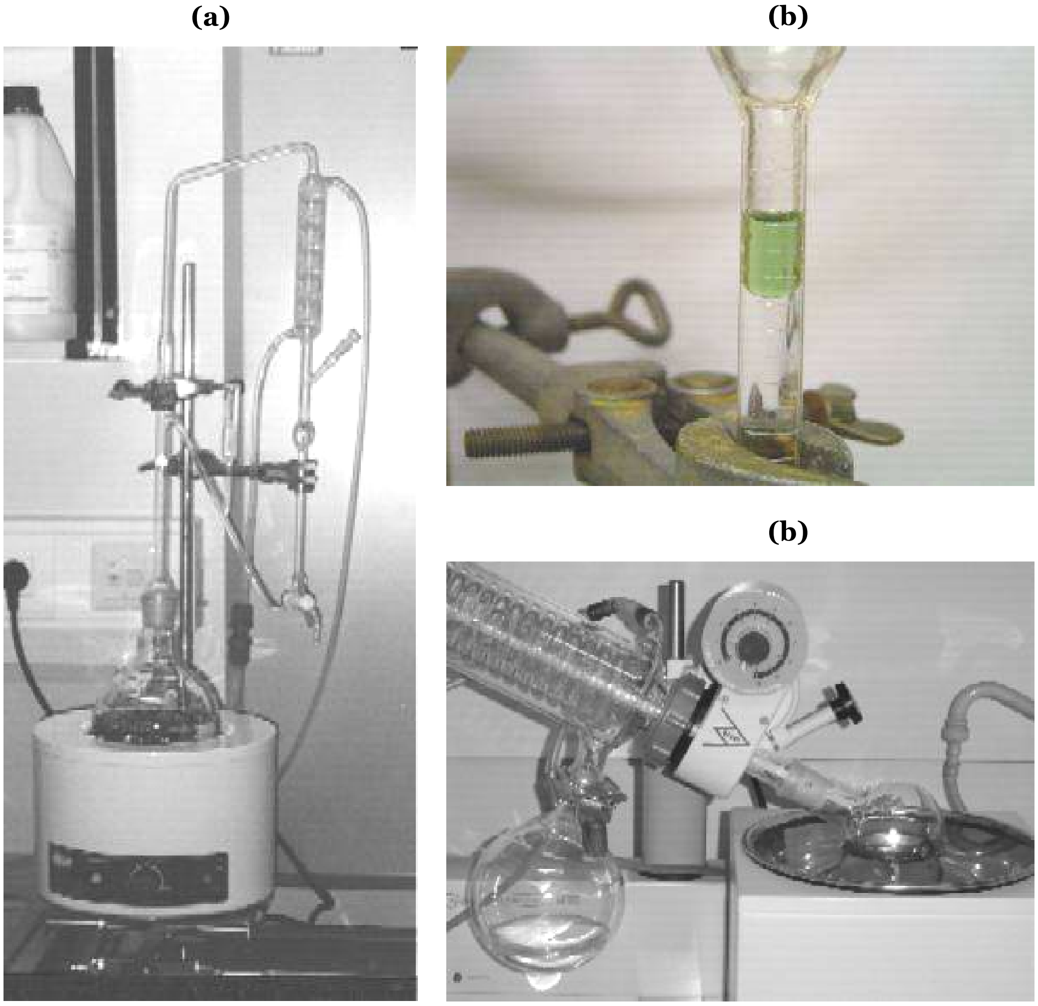


Figure 3.8 Equipment used for isolating the essential oil: (a) Clevenger apparatus;(b) Collection of essential oil; (c) rotary evaporator under reduced pressure.

Three kinds of extracts from solvent extraction with hexane, ethanol and diethyl ether were also obtained. The fresh plant material (30 g) was initially extracted with each solvent (250 ml) during 1h. The extract was then filtered and concentrated by a rotary evaporator under reduced pressure (Figure 3.8-c) at 50 °C, 65 °C and 20 °C, respectively. The solid residue (the concrete) was extracted with ethanol and again filtered under reduced pressure, resulting finally in the absolute.

Each isolation procedure was performed twice. The oils and absolutes were stored in glass vials and in a cold-storage room prior to their analysis.

Gas chromatography/Mass spectrometry (GC/MS)

Gas chromatography GC/FID and GC/MS were carried out in parallel, using a Varian CP-3800 instrument equipped with 2 split/splitless injectors, 2 CP-Wax 52 CB bonded fused silica polar columns (50 m x 0.25 mm, 0.2 micron m film thickness), a Varian FID detector and a Varian Saturn 2000 MS ion-trap mass spectrometer, both controlled by Saturn 2000 WS software (Figure 3.9). The oven temperature was programmed isothermal at 50 ºC for 5 min, then increased from 50 - 200 ºC at 2ºC/min and in the end held isothermal for 40 min. The injectors were set at 240 ºC, with a split ratio of 1/50 for FID and 1/200 for MS. The FID detector was maintained at 250 ºC. The sample volume injected was 1 µL. The carrier gas was helium He N60, at a constant flow rate of 1 ml/min. There were made 3 injections per sample and the sample GC vial was kept in a cold-storage room between injections.

Component identification and quantification

The essential oil composition was expressed in percentage values calculated directly from GC peak areas and in a base without solvent. The areas were used without applying correction factors.

Figure 3.9 Gas chromatography equipment Varian CP-3800, with FID (Varian) and MS (Saturn2000) detectors.

The linear retention indices (LRI) were determined relative to the retention times obtained for a standard mixture containing a series of n-alkanes C8-C20 (Fluka, Spain), as follows:

$$LRI_x = 100n + 100\frac{t_{Rx} - t_{Rn}}{t_{Rn+1} - t_{Rn}} \qquad (3.1)$$

where x is component eluting at retention time t_{RX}; n is the carbon number of the n-alcane eluting before x at time t_{Rn}; n+1 is the carbon number of the n-alcane eluting after x at time t_{Rn+1}.

The component identification was made by comparison of mass spectra using NIST98 spectral library, pure reference components (own laboratory library) and literature data. The identification was also confirmed by comparison of retention indices with those of injected pure reference components and published values obtained on the same type of polar column.

The composition of each sample was determined as the average of the three GC injections.

3.2.5 Results and discussion

The essential oil yield calculated from fresh plant material was 0.1 - 0.2%.

The main components identified in the Portuguese-grown geranium oil (which constitute 76% of the total composition) are shown in Table 3.1 and in chromatogram in Figure 3.10.

Germacrene-D was only tentatively identified based on NIST library search and on the fact that it has been already reported in geranium oil (Demarne and van der Walt, 1989; Lawrence, 1984; Walt and Demarne, 1988). The identification of guaia-6,9-diene was made by comparison with published MS spectra (Steele *et al.*, 1998), presented in Figure 3.11.

Comparing the composition of the obtained geranium oil with the data published by Teisseire (Teisseire, 1987) for the three kinds of commercial geranium oils, it was concluded that the composition was intermediate between the Bourbon and Chinese oils, with the same content of guaia-6,9-diene and the absence of 10-epi-γ-eudesmol, as can be seen in Figure 3.12. The citronellol/geraniol ratio was 3.3, which was a value closer to the Bourbon (1.2) than to China type (8.3). The main composition of our geranium oil was also very similar with the oil obtained from geranium grown in

Table 3.1 Main components identified in Portuguese-grown geranium essential oil.

N	Component	LRI*	Composition (%)
1	rose oxide	1339	0.5
2	isomenthone	1471	5.6
3	linalool	1537	2.7
4	guaia-6,9-diene	1591	5.9
5	citronellyl formate	1609	13.2
6	germacrene-D **	1688	2.4
7	geranyl formate	1692	5.5
8	citronellol	1754	26.9
9	geraniol	1832	8.1
10	geranyl tiglate	2067	3.3
11	2-phenylethyl tiglate	2120	1.8

* Linear retention indices (LRI) determined in a CP-Wax 52 CB column
** Tentatively identified: m/z(rel.int.) - 204 (M+) (25), 189 (5), 161 (100), 147 (9), 133 (30), 119 (37), 105 (51), 91 (41), 89 (23), 67 (13), 55 (10), 39 (37)

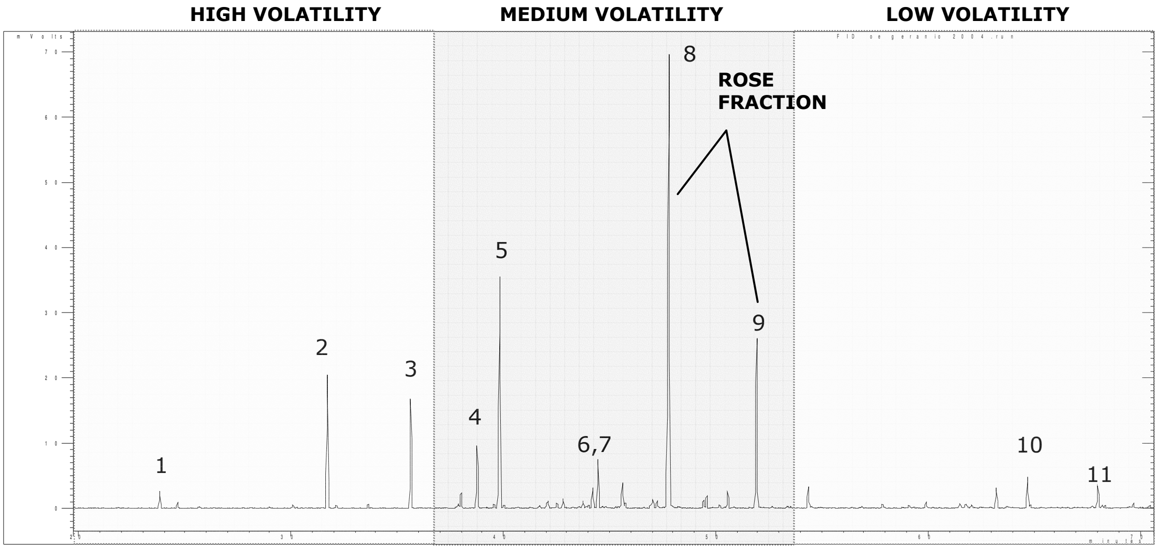


Figure 3.10 GC/MS chromatogram of Portuguese-grown geranium oil obtained from fresh green foliage of Pelargonium sp. (see table 3.1 for correspondence number-component).

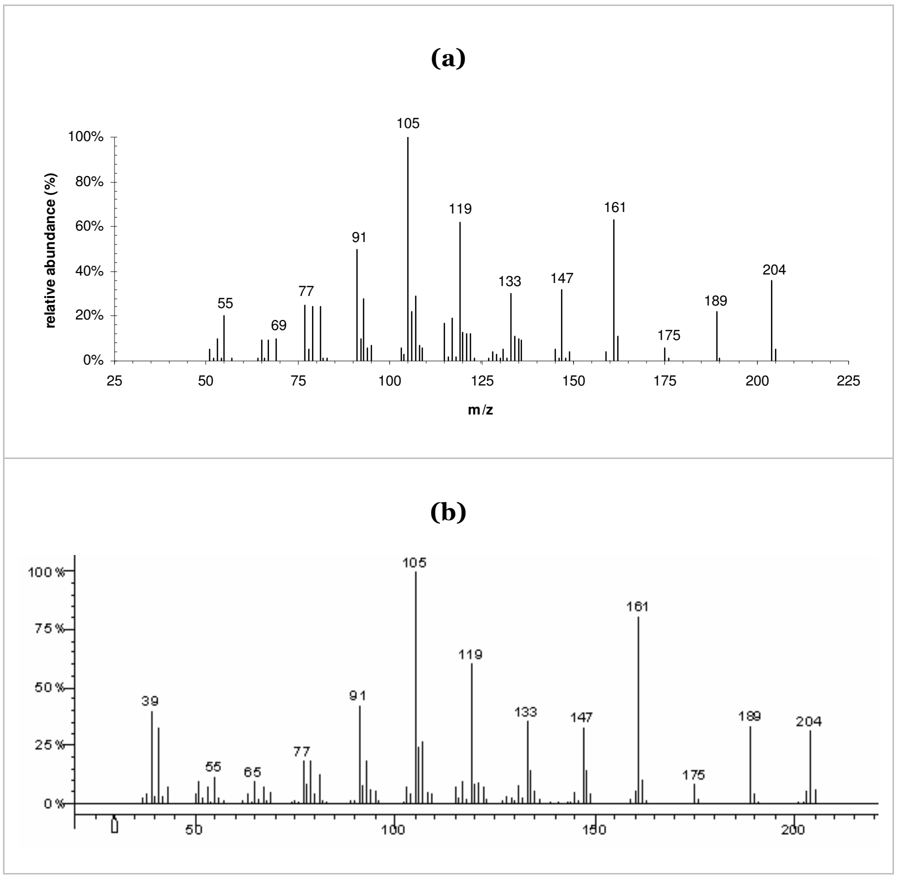


Figure 3.11 MS spectra of guaia-6,9-diene: a – published (Steele et al., 1998); b - obtained in this laboratory.

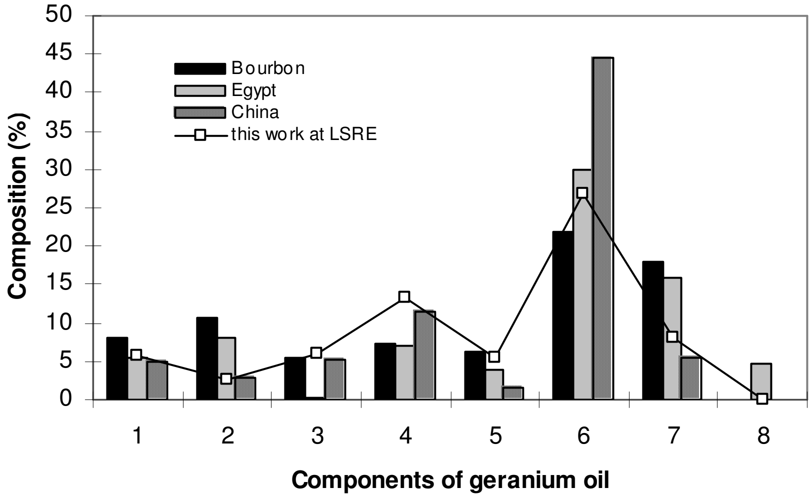


Figure 3.12 Comparison of composition of Portuguese-grown geranium oil with the three kinds of commercial geranium oils (data on columns from Teisseire (Teisseire, 1987) and data points obtained in this work). 1. isomenthone; 2. linalool; 3. guaia-6,9-diene; 4. citronellyl formate; 5. geranyl formate; 6. citronellol; 7. geraniol; 8. 10-epi-γ-eudesmol.

Reúnion Island (*cv. Rosé*) described by Demarne and van der Walt (Demarne and van der Walt, 1989), except for the lower concentration of geraniol.

When comparing our analysis with other Portuguese characterization studies, the composition approached that obtained by Seabra and Carmo (Seabra and Carmo, 1989) except for the absence of 10-epi-γ-eudesmol characteristic of the African type (Figure 3.13). Therefore, the present work reports the characterization of a new geranium oil.

Influence of preparation and condition of plant material

The influence of preparation and condition of the plant on the composition of the oil is shown in chromatograms in Figure 3.14 and Table 3.2. The geranium oil obtained from yellow leaves presented a content of rose oxide and isomenthone (more volatile) higher than the oil obtained from green leaves, increasing their concentration 6 and 1.5 times, respectively. On the other hand, the content of geraniol and its esters and of linalool decreased 40 to 70%, while citronellol and its esters maintained their levels. The concentration of guaia-6,9-diene decreased significantly. The intensification of rose odour that is reported when using yellow leaves is therefore due to the high content of rose oxide.

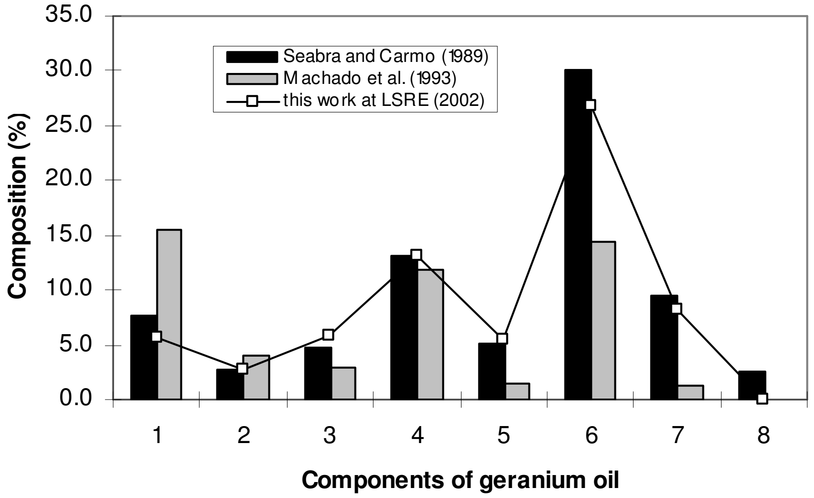


Figure 3.13 Comparison of composition Portuguese-grown geranium oil with other Portuguese geranium oils (Machado et al., 1993; Seabra and Carmo, 1989). 1. isomenthone; 2. linalool; 3. guaia-6,9-diene; 4. citronellyl formate; 5. geranyl formate; 6. citronellol; 7. geraniol; 8. 10-epi-gamma -eudesmol.

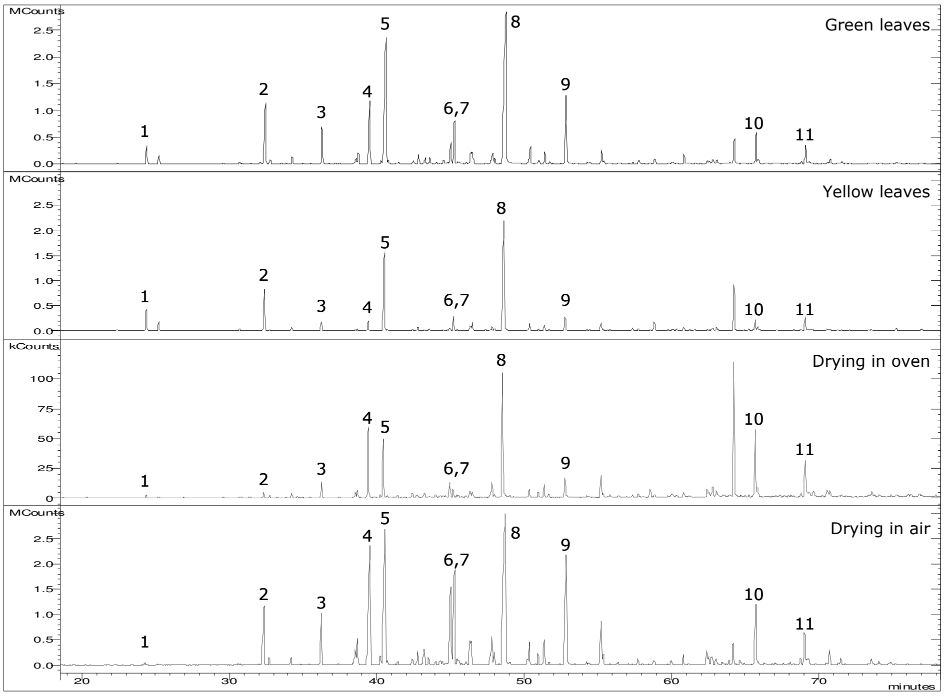


Figure 3.14 GC/MS chromatograms of geranium oil obtained with different plant preparation and condition.

Table 3.2 Peak area percentage composition of geranium oil obtained with different plant preparation and condition.

Component	Fresh leaves		Dried leaves	
	green	**yellow**	**oven**	**air**
rose oxide	0.5	2.9	0.2	0.1
isomenthone	5.6	8.4	0.9	4.5
linalool	2.7	1.4	2.0	4.0
guaia-6,9-diene	5.9	1.2	5.8	6.2
citronellyl formate	13.2	14.0	5.2	11.1
germacrene-D **	2.4	0.4	3.1	2.7
geranyl formate	5.5	2.5	2.2	5.5
citronellol	26.9	27.6	18.8	25.1
geraniol	8.1	2.5	6.5	10.6
geranyl tiglate	3.3	1.7	7.0	3.5
2-phenylethyl tiglate	1.8	2.8	3.9	1.9
Total	75.9	65.4	55.7	75.1

Drying the leaves in an oven prior to extraction resulted in an oil that was poorer in all the considered components except for geranyl and 2-phenylethyl tiglates, the less volatile components. In fact, the contents of isomenthone decreased more than 80%, while the concentration of the tiglates doubled. On the other hand, the air-dried geranium oil was found to contain the highest content of linalool and geraniol, having though approximately the same amount of citronellol and esters as using fresh geranium leaves.

In conclusion, the quality of our geranium oil improved with air-drying because the ratio citronellol/geraniol decreased from 3.3 to 2.3, being closer to Bourbon type. When yellow leaves are included, the olfactory quality should also improve due to the higher content of rose oxide.

Influence of extraction method

The traditional processes used to obtain the essential oil from the plant, namely hydrodistillation and volatile solvent extraction, are compared in Figure 3.15 and Table 3.3. The composition of the hexane extract herein obtained was in agreement with the results reported by Guerere et al. (Guerere *et al.*, 1985), in particular regarding the trace amount of linalool and the higher contents of geraniol found in the extract.

The composition of the absolutes obtained from hexane and diethyl ether extractions were very similar with linalool only present in trace amounts, isomenthone and citronellyl formate in half of the quantity, 20% less of citronellol and a higher content of geraniol and tiglates when compared to the oil composition. They differed in the higher content of rose oxide in the absolute from diethyl ether extract, what justify the better quality imparted to this absolute. Ethanol extraction resulted in an opaque absolute, with a high level of non-volatiles. The higher percentage of linalool observed in this ethanol extract, associated to a decrease in geraniol content, resulted from the conversion of geraniol to linalool due to the use of a higher temperature and longer time to remove the solvent, during the extraction procedure, as explained by Guerere et al. (Guerere *et al.*, 1985).

Influence of time of harvest

The yield and composition of Portuguese-grown geranium oil and absolute of hexane extract obtained

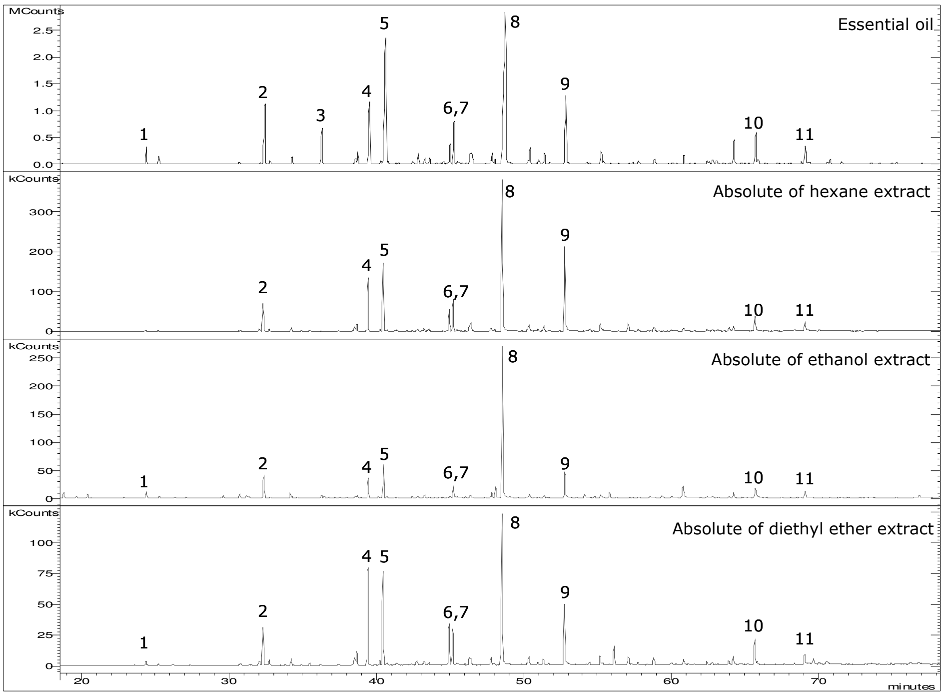


Figure 3.15 GC/MS chromatograms of the oil and absolutes obtained from different extracts of geranium leaves.

Table 3.3 Peak area percentage composition of the oil and absolutes obtained from different extracts of geranium leaves.

	Hydrodistillation	**Solvent extraction**		
Component	**Geranium Oil**	**Hexane**	**Ethanol**	**Diethyl ether**
rose oxide	0.5	0.0	0.7	0.2
isomenthone	5.6	2.1	2.2	2.3
linalool	2.7	0.0	0.5	0.0
Guaia-6,9-diene	5.9	5.5	2.1	6.4
citronellyl formate	13.2	6.0	4.0	6.5
germacrene-D **	2.4	3.2	0.2	3.7
geranyl formate	5.5	4.7	1.7	4.7
citronellol	26.9	21.3	24.0	20.8
geraniol	8.1	10.8	5.0	9.2
geranyl tiglate	3.3	3.3	3.3	4.4
2-phenylethyl tiglate	1.8	2.2	1.2	2.2
Total	75.9	59.2	44.8	60.5

from plant harvested in the summer (June/July) was compared with the previous results obtained for the plant collected in autumn (October/November). The major difference was that the plant was in flower. The composition of this oil and absolute is shown in Figure 3.16 and Table 3.4.

The essential oil yield was 0.24%, higher than the yield obtained before. The composition was also different, being the summer oil richer in the more volatile fragrant components rose oxide, isomenthone, linalool, citronellol and geraniol, whilst the content in the little volatile geranyl tiglate and phenylethyl tiglate is lower comparing with the composition of the essential oil from the plant collected in autumn. The components that increased most their content in the oil were linalool (150%), geraniol (36%) and isomenthone (30%).

Comparing the composition of the absolutes of the hexane extracts, the composition has changed in the same way, but with more significant increase in the amount of isomenthone that is 3 times higher, geraniol that doubled its amount and citronellol that increased 56%.

Therefore the odour of the oil has an accentuated fresher character due to the higher content in isomenthone, as well as is more floral with a more intense rosy aspect because of the higher amount of geraniol/citronellol. This stronger floral character is explained by the fact of the plant being in flower and having higher content of essential oil.

Organoleptic characteristics

From the organoleptic point of view, the geranium oil produced was transparent light-green coloured, with an initial green odour that evolved to a sweet-floral aroma, slightly fresh. This oil has the same colour as the Bourbon geranium oil, which is one more indication of the similarity of Portuguese-grown geranium oil to this better quality essential oil.

The absolutes were green-brownish, dark green and olive-green coloured from hexane, ethanol and diethyl ether extracts, respectively. The odour was intensely green at first, evolving afterwards to a sweet-floral aroma. In the case of ethanol extract, the odour changed from unpleasant to somewhat balsamic odour. All the absolutes stained the smelling strip, what means that they contained non-volatiles. The ‘boiled’ *off notes* were noticed in the hydrodistilled oils.

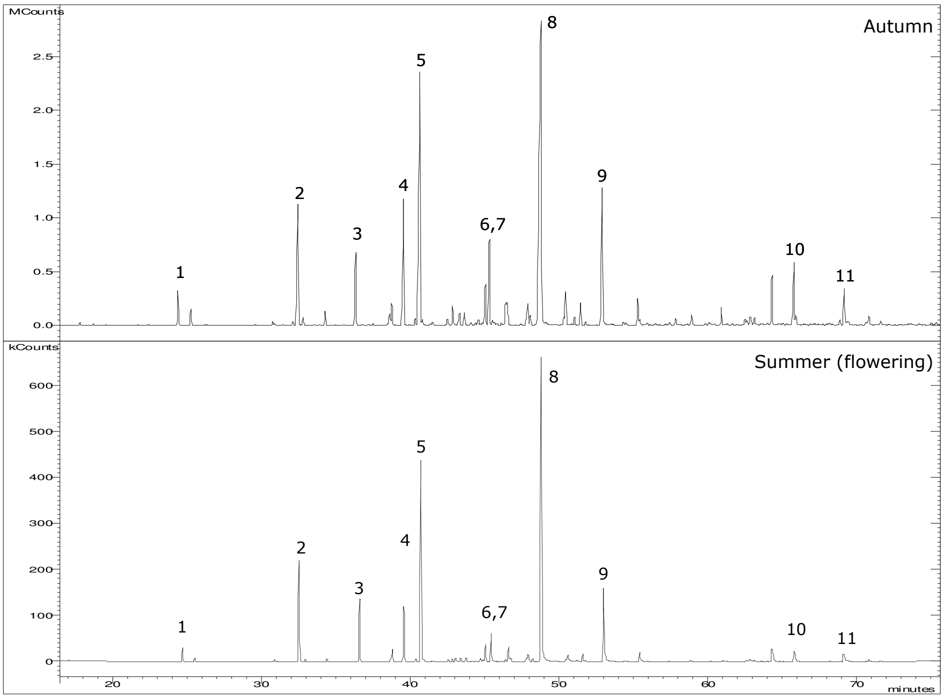


Figure 3.16 GC/MS chromatograms of geranium oil and absolute of hexane extract obtained from green leaves and flowers from plant collected in the Summer.

Table 3.4 Peak area percentage composition of geranium oil and absolute of hexane extract obtained from green leaves and flowers from plant collected in the Summer.

N	Component	Essential Oil	Absolute
1	rose oxide	0.7	0.0
2	isomenthone	7.3	6.3
3	linalool	6.8	0.0
4	guaia-6,9-diene	5.3	6.9
5	citronellyl formate	13.6	10.1
6	germacrene-D **	1.5	3.2
7	geranyl formate	3.0	5.1
8	citronellol	30.4	33.2
9	geraniol	11.0	21.4
10	geranyl tiglate	2.5	2.8
11	2-phenylethyl tiglate	1.4	1.5
	TOTAL	83.3	90.5

3.2.6 Conclusions

The chemical composition of the Portuguese-grown geranium essential oil (*Pelargonium hybrid*) cultivated in the North of Portugal, which also grows wild, was examined. It was found that the geranium oil herein obtained has characteristics intermediate between Bourbon and China types, more resembling the better quality of Bourbon type, with good olfactory quality and presenting characteristics different from those previously studied. The essential oils of fresh/dried and green/yellow geranium leaves were compared. Solvent extraction methods were also employed and the composition of the respective absolutes was determined and compared with the essential oil. It was concluded that using air-dried plant and yellow leaves improved the quality of geranium oil. Moreover, the absolute obtained from a diethyl ether extract had the best organoleptic properties of the extracts studied.

This work on the characterization of the Portuguese-grown geranium, extracted by the traditional methods of hydrodistillation and organic solvent extraction, will be the basis for Chapter 4, namely the study of the supercritical fluid extraction of the essential oil from this aromatic plant.

3.3 Cistus (*Cistus ladanifer*) essential oil growing wild in Portugal

Cistus ladanifer, also known as rockrose or labdanum, is a resinous and extremely fragrant bush that grows wildly in the Mediterranean region (Portugal, Spain, France, Morocco). The plant is easily recognizable by its white flowers with a crimson spot in the base of the petals and by its fragrant resin (Figure 3.17). The resin is produced in glandular hairs during the summer months, protecting the plant from dehydration.

Labdanum oil obtained from the resin is an important raw material for perfumery industry since it constitutes an important vegetal alternative to ambergris, which is a rare ingredient obtained from a protected animal source – whales – and is essential for composing oriental perfumes. Moreover, it provides starting materials for manufacturing synthetic ambers (Groom, 1997).

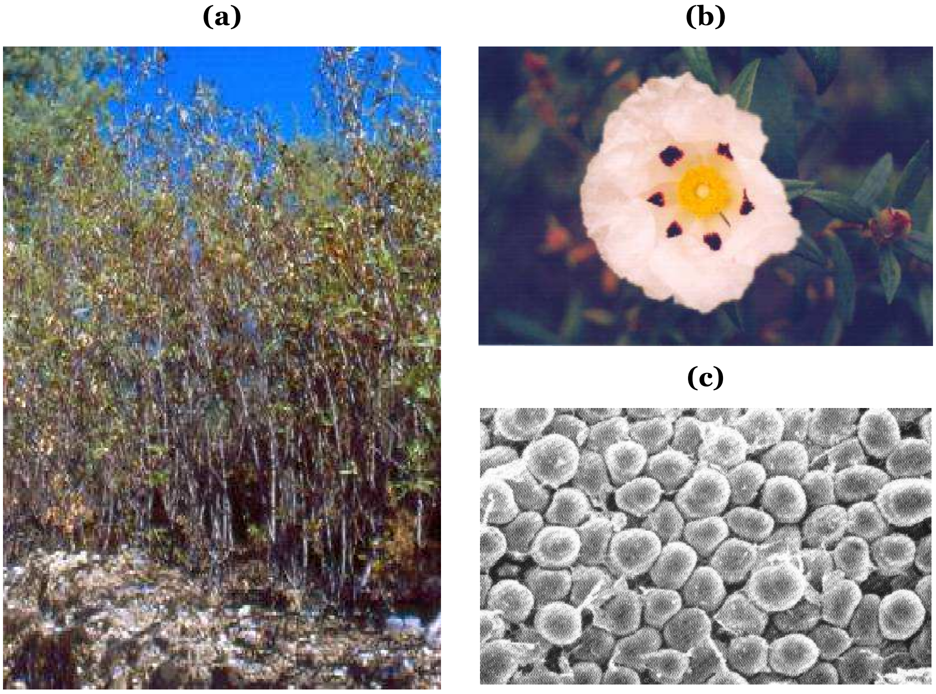


Figure 3.17 Rockrose plant (Cistus ladanifer L.): (a) bush in the wild; (b) flower and (c) trichomes that contain the essential oil (source of this photo: Borges, 1987).

In Portugal, rockrose grows in the wild in the mountain regions of Beira Interior through Algarve and all over Alentejo, preferring the places that are dry, warm, sunny and sheltered from winds. The plant is considered a plague by the farmers and so the cost of the plant material is considerably low (Frazão *et al.*, 1984)

There are several perfumery materials obtained from cistus, namely (Frazão *et al.*, 1984):

- gum - dark and almost solid, obtained by boiling the dry plant in water and skimming the gum from the surface, which is left to dry;
- resinoid – dark and viscous extract obtained by alcoholic extraction of gum and therefore soluble in ethanol;
- labdanum oil – essential oil obtained by hydrodistillation of the gum;
- concrete – extract obtained by solvent extraction of the plant and concentrated by removal of solvent;
- absolute – extract obtained by alcoholic extraction of the concrete;

- cistus oil – essential oil obtained by hydrodistillation of the plant (fresh or dry).

Spain is the main world producer of labdanum products (Bauer *et al.*, 1997; Simon-Fuentes *et al.*, 1987), which annual production is approximately 20 tonnes (Pybus and Sell, 1999).

3.3.1 Organoleptic characteristics

The resin exuded by the plant has a pleasant, woody and balsamic odour that becomes almost animalic when the odour is very intense at the summer time. The labdanum oil is the most precious perfumery material from rockrose, being a golden coloured liquid with a very strong odour that is used to compose the base note of chypre perfume compositions, imparting a balsamic and leathery character. The typical odour is due to the presence of components resulting from oxidative degradation of diterpenes with labdane skeleton existing in labdanum gum as the main components (Bauer *et al.*, 1997).

On the other hand, cistus oil constituted mainly by monoterpenes (Bauer *et al.*, 1997), so having a very “terpenic” odour, with strong impact top notes that are not very resembling of the scent of the plant (Frazão *et al.*, 1984).

3.3.2 Perfumery and cosmetic applications

Labdanum is a valuable raw material in perfumery industry as a natural fixative and for composing amber and leathery notes, being used in 33% of all modern quality perfumes and 20% of all quality perfumes for men (Groom, 1997). Labdanum oil is used in fine perfumery due to its light colour, imparting exotic nuances to the fragrance character. It is a main ingredient for the composition of the chypre accord that blends bergamot, rose, jasmine, oakmoss, patchouli and labdanum.

In recent perfumes, labdanum is used in Acqua di Gio for men by Armani (1996) to impart woody notes, and more recently it was used in Rush for men by Gucci (2000), which composition is shown in Figure 3.18-a. In women’s fragrance, this ingredient is also employed in oriental perfumes such as in Theorema by Fendi (1999) (see Figure 3.18-b).

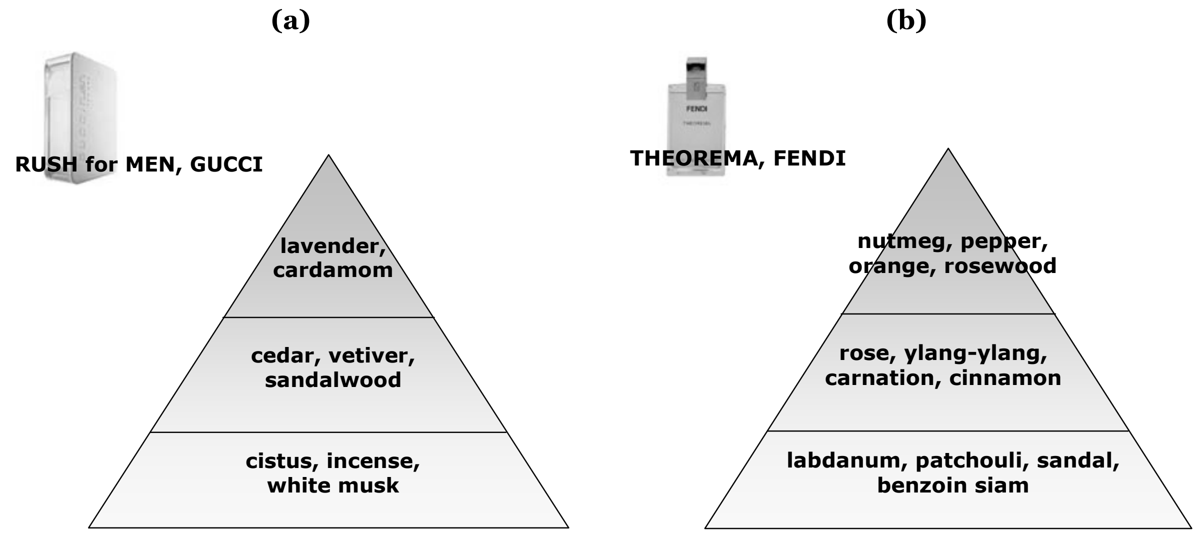


Figure 3.18 Composition of two perfumes with labdanum/cistus note: (a) men's fragrance, Rush for man, Gucci (2000); (b) women's fragrance,Theorema, Fendi(1999).

The concrete is mainly applied directly in soaps, working as fixative and imparting a balsamic odour. On the other hand, absolutes are used as bases for perfume compositions, contributing to the balsamic character (Frazão *et al.*, 1984).

3.3.3 Review on rockrose

The main composition of the cistus oil published in the literature is presented in Table 3.5.

These compositions differ significantly although it is possible to consider two groups: Group A with α-pinene as principal component and Group B with viridiflorol in higher quantity and having a low content of α-pinene. Group A is the most common.

Simon-Fuentes et al. (Simon-Fuentes *et al.*, 1987) identified by GC/MS the main components in the neutral volatile fraction of a commercial cistus oil produced in Spain, where the highly volatile monoterpenes α-pinene, camphene and p-cymene constitute almost 50% of the composition. The norterpene 2,2,6-trimethylcyclohexanone was also a major component (5.7%) that has been associated to the fragrance of the plant (Ramalho *et al.*, 1999).

Table 3.5 Percentage composition of cistus essential oils found in the literature, comparing the most common components.

Component	GROUP A					GROUP B	
α-pinene	35.0	5.3-25.6	56.0	39.0	24.0	0.4	2.3
camphene	10.0	0.9-6.5	1.9	2.1	3.1	-	-
limonene	2.3	0.2-0.8	1.7	1.7	1.5	-	-
p-cymene	4.0	1-4.8	1.2	1.7	1.2	0.4	0.6
2,2,6-trimethylcyclohexanone	5.7	4.5-11.9	2.5	0.9	6.0	0.8	1.4
α-campholenic aldehyde	0.8	-	2.3	-	3.0	0.8	-
bornyl acetate	3.7	1.9-3.7	2.1	3.1	-	2.4	2.1
terpinen-4-ol	1.8	1-2.6	0.8	1.1	3.6	0.7	3.1
allo-aromadendrene	1.2	-	0.7	1.9	-	1.0	-
trans-pinocarveol	3.4	-	0.8	1.9	3.5	2.5	7.0
borneol	1.0	0.5-2.5	-	0.8	3.0	1.5	1.4
myrtenol	-	1.1-2.4	0.6	0.4	1.3	-	1.9
ledol	-	6.6-13.8	0.7	3.3	1.8	0.4	6.6
viridiflorol	-	-	2.9	11.8	6.3	12.8	21.3
Reference	[1]	[2]	[3]	[4]	[5]	[6]	[7]

References:1. (Simon-Fuentes *et al.*, 1987); 2. (Regino *et al.*, 1987); 3. (Vernin, 1992) refered by (Lawrence, 1999b); 4. (Mariotti *et al.*, 1997): bulk sample obtained by hydrodistillation in industrial apparatus; 5. (Robles *et al.*, 2003); 6. (Mrabet et al., 1997) refered by (Lawrence, 1999b); 7. (Mariotti *et al.*, 1997): average composition of cistus oil from plants of group I.

Ramalho et al. (Ramalho *et al.*, 1999) studied by GC/MS and GC/Sniffing the aromatic volatile components existing in the leaves of *C. ladaniferus* from Douro region (Portugal) that are related to the aroma quality of Porto wine. The components 2,2,6-trimethylcyclohexanone (rock-rose leaf odour), acetophenone, 2-phenylethanol (floral aroma) and in small amounts 2,6,6-trimethylcyclohex-2-enone (woody balsamic-like odour) were pointed out as the most important odoriferous constituents.

Peyron et al. (Peyron *et al.*, 1986) made a detailed description on the industrial use of cistus cultivated in Corsica, including data on botanical, cultivation, extraction technologies and essential oil characteristics.

Mariotti et al. (Mariotti *et al.*, 1997) characterized the chemical composition of cistus oil isolated from leaves and stems of *Cistus ladaniferus* of Spanish origin but grown in Corsica, using GC/FID and 13C-

NMR. The authors identified 45 components, of which the main components were pinene (39%), viridiflorol (11.8%), ledol (3.3%) and bornyl acetate (3.1%). A study of chemical variability showed that 20 samples of Cistus oil displayed significant variations in their composition. They were divided in six groups, where in Group III to VI the major component was α-pinene (respectively 20.4-24.2%, 29.8-33.0%, 36.8-40.3% and 44.1-47.4%). Group I presented a considerable higher content of viridiflorol (20-22.6%) and a lower amount of α-pinene (2.1-2.5%), and the remaining Group II was intermediate between Group III and Group I with α-pinene and viridiflorol in high amount (respectively 11.1-16.4 and 9.5-10.7%).

Mrabet et al., cited by Lawrence (Lawrence, 1999b) characterized the oil of two varieties of cistus grown in Morocco, which also had viridiflorol as the main constituent (12.81-20.68%) and a very low content of α-pinene (0.30-0.37%). The variety maculatus contained an unusual high level of camphor (2.92%) and a low content of 2,2,6-trimethylcyclohexanone (0.75%).

Weyerstahl et al. (Weyerstahl *et al.*, 1998) described with great detail the composition of a commercial labdanum oil. The composition of the oil was determined by GC/MS and NMR analysis. The components found in major quantity were ledene (9.3%), viridiflorol (4.3%) and cubeban-11-ol (4.1%). The characteristic components of rockrose 2,2,6-trimethylcyclohexanone (1.3%) and 2,2,6-trimethylcyclohex-2-enone (0.3%) were found in smaller amounts. One of the most interesting aspects of their work was the identification of olfactory key components for the amber scent, namely 11-nor-driman-8-ol (0.3%, powerful woody-amber), ambrox (1.4%, strong dry amber), α-ambrinol (0.2%, warm animal amber), drimenone (0.1%, tobacco, amber), 6-acetoxy-nor-drimenone (0.2%, soft warm, strong amber, woody), oxoambrox (0.1%, soft animal amber, like ambrox), sclareolide (0.2%, weak fine amber, woody-resinous tone), labdenols (weak amber-animal) labd-8(17)-en-15-ol (0.4%), labd-7-en-15-ol (0.4%) and labd-8-en-15-ol (0.3%). Sclareol oxide (0.2%) was also identified as a minor component of labdanum oil, being that component an important intermediate for the synthesis of ambergris-odorants for perfumery industry and it is usually obtained from clary sage oils (Ohloff, 1994).

Ohloff (Ohloff, 1994) pointed out some components responsible for labdanum odour, namely ambrox and 6-oxo-isoambrox for amber scent; phenols for the smoky leathery notes; methyl b-orcinol carboxilate for powdery moss character; rose oxides, 2-phenylethyl alcohol, 9-oxodamascone, ledol and viridiflorol for floral notes; tagetone and (Z)-ocimenone for herbaceous odour; and raspberry ketone and its methoxy-derivative impart a fruity scent.

Rincón et al. (Rincón *et al.*, 2000) established the best operation conditions of supercritical CO2 extraction (SFE) of rockrose growing in Spain. The main components of the supercritical extract obtained at optimum conditions were camphor (22%), α-pinene (19.5%), γ-terpineol (4.6%), borneol (4.4%), camphene (4.2%), thymol (4.2%), γ-terpinene (4.1%) and p-cymene (4,0%). SFE is an interesting alternative to conventional extraction methods of essential oil from plants since it prevents thermal degradation and solvent pollution of the extract. Indeed the authors found that the odour of the supercritical extract was quite similar to the plant, while the steam-distilled oil had a slightly sweeter odour.

More recently, Robles et al. (Robles *et al.*, 2003) compared the composition of the oils of two varieties of *Cistus ladanifer* growing in France: *C. ladanifer var. maculatus* and *C. ladanifer var. albiflorus*. The two varieties can be distinguished by the difference in the chemical composition of the following eight components: α-pinene, β- pinene, γ-terpinene, verbenone, benzaldehyde, p-cymen-8-ol, ledol and viridiflorol.

Regino et al. (Regino *et al.*, 1987) studied the cistus grown in Portugal. They considered the variation of composition and yield of cistus oil along the year, during storage and using plants from three different geographical sources. The composition changed but the main components were always α-pinene (5.34-25.56%), 2,2,6-trimethylcyclohexanone (4.46-11.88%) and ledol (6.59-13.78%). The higher yields were obtained with plant harvested in August and September. The authors also compared the oil with the extracts of benzene and hexane-dichloroethane obtained from labdanum concrete, which showed gas chromatographic profiles very similar to the oil but with lower contents or even absence of some components, such as α-pinene and ledol.

Lawrence (Lawrence, 1986; 1990; 1999b) published some reviews on cistus and labdanum isolates.

In the present work, the main composition of *Cistus ladanifer* oil obtained from plants growing in Portugal is reported, highlighting the interesting components from the perfumery point of view. A comparison with available commercial samples is also performed.

3.3.4 Materials and methods

Plant material

Cistus ladanifer was collected from two sites, in July-August 2001, after the flowering season. The major quantity was brought from the wild, where the plant was growing in the mountainous region of the center-interior of the country (site 1). A smaller amount was harvested from a cultivated plant in the North of Portugal (site 2) that was propagated from a wild plant found in the dry plain region in the South of Portugal.

Oil isolation

The cistus oil was obtained by hydrodistillation of fresh and dry plant material (20 g), using leaves and small branches, in a Clevenger-type glass apparatus. The plant/water proportion was 1:30 and the distillation time was 2 hours. The dry cistus oil obtained was the cumulative oil of several hydrodistillations. The oils were stored in a glass flask and kept in a freezer prior to their analysis.

Gas chromatography/Mass spectrometry (GC/MS)

The procedure has already been described for geranium (section 3.2.4).

Component identification and quantification

The procedure has already been described for geranium (section 3.2.4).

The identification was also confirmed by comparison of retention indices with those of injected pure reference components and published values obtained on the same type of polar column (Mariotti *et al.*, 1997; Weyerstahl *et al.*, 1998).

3.3.5 Results and discussion

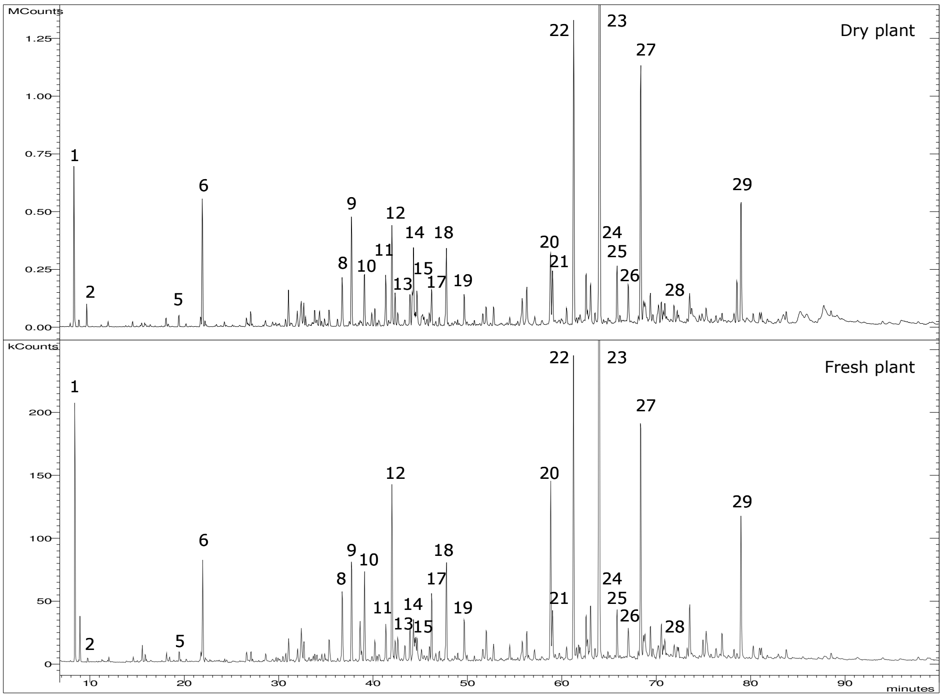
The oil yield calculated from dry plant material was 0.2-0.3%.

The main composition of the oils of cistus growing in two different sites in Portugal is shown in Figure 3.19 and Table 3.6. Most of the components identified have been previously reported as constituents of this oil. The composition is considerably more complex (around 250 components with more than 0.1% and exceeding 400 components when including those in trace amounts).

Therefore there was considered this group of components in order to allow comparing and distinguishing the oils. They were also representative of the several kinds of components existing in these cistus oils, namely containing: high volatility monoterpenes hydrocarbons (components 1 to 5, 7) and oxygenated (component 6), medium volatility components sesquiterpenes (components 11, 14, 18) and oxygenated monoterpenes (components 8, 9, 10, 12, 13, 15 and 19), and low volatility oxygenated components (components 20 to 29), including those contributing to amber scent (components 26 and 29).

Viridiflorol, globulol, an unknown sesquiterpene alcohol (component 27) and the diterpene alcohol 15-nor-labdan-8-ol were the constituents existing in higher quantity in the cistus oils from both sites. The component 15-nor-labdan-8-ol was tentatively identified by comparison with MS data published by Weyerstahl et al. On the other hand, the contents of α-pinene and other volatile monoterpenes were relatively low, so the oil here studied was similar to Group B cistus oils. However the content in 2,2,6-trimethylcyclohexanone, characteristic of rockrose odour, was 2 to 3 times higher in our oil. Another component found with olfactory interest is ambrox, with an amber scent resembling ambergris and very valuable to the perfumery industry (Bauer *et al.*, 1997). It is noteworthy that ambrox and sclareol oxide have already been referred as labdanum constituents (Weyerstahl *et al.*, 1998) but not as main components of the cistus oil, as this work reports. Moreover, the component caryophyllene oxide having a woody slightly ambergris scent (Bauer *et al.*, 1997) was also present in higher amount than usual. When comparing with the previous works, including the one on Portuguese cistus oil (Regino *et al.*, 1987), the composition of our oil is quite different. Therefore, this work reports a novel composition of cistus oil richer in amber-like components.

SITE 1



SITE 2

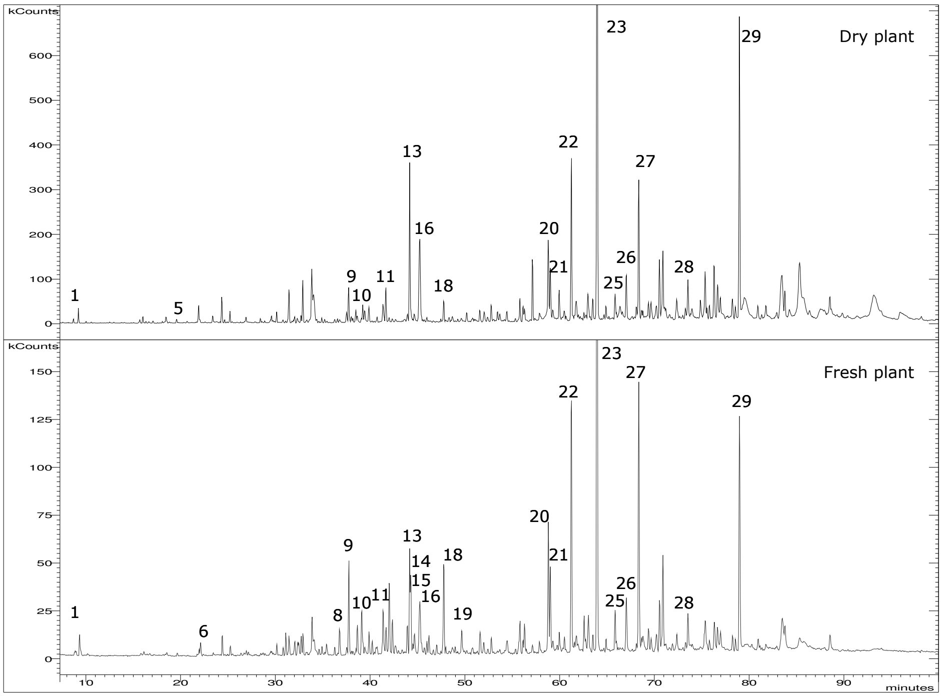


Figure 3.19 GC/MS chromatograms of cistus oil obtained from dry and fresh plant, collected at two different sites (see table 3.6 for correspondence number-component).

Table 3.6 Percentage composition and linear retention indexes of components identified in the essential oil of Cistus ladanifer growing in 2 sites in Portugal, hydrodistilled from dry and fresh plant.

			Site 1		**Site 2**	
N	**Component**	**LRI***	**Fresh**	**Dry**	**Fresh**	**Dry**
1	α-pinene	1015	2.1	4.5	0.1	0.2
2	camphene	1055	0.3	0.1	0.0	0.0
3	β-pinene	1102	-	-	-	-
4	limonene	1190	-	-	-	-
5	p-cymene	1259	0.2	0.3	0.1	0.1
6	2,2,6-trimethylcyclohexanone	1302	2.8	2.0	0.0	0.3
7	p-cymenene	1424	-	-	-	-
8	pinocarvone	1545	1.1	1.7	0.0	0.7
9	bornyl acetate	1562	1.6	1.5	0.7	1.4
10	terpinen-4-ol	1585	1.0	1.5	0.3	0.8
11	allo-aromadendrene	1624	0.8	0.7	0.5	0.7
12	trans-pinocarveol	1636	2.1	3.8	0.0	0.0
13	(Z)-ocimenone	1673	0.6	0.2	4.0	2.2
14	(E)-ocimenone	1692	-	-	3.6	1.9
15	viridiflorene	1677	1.3	1.0	0.0	1.0
16	borneol	1682	0.7	0.5	0.1	0.5
17	unknown (a)	1710	0.7	1.3	-	-
18	δ-cadinene	1739	1.0	1.6	0.4	1.1
19	myrtenol	1773	0.7	1.1	0.0	0.7
20	caryophyllene oxide	1950	1.8	3.6	1.7	2.5
21	$C_{15}H_{26}O$ sesquiterpene alcohol (b)	1954	1.1	0.5	0.7	0.9
22	globulol	2000	5.0	4.3	3.1	4.0
23	viridiflorol	2043	17.4	15.1	13.6	14.9
24	d-verbenone	2059	0.5	0.9	-	0.1
25	(-)- spathulenol	2069	0.8	0.8	0.5	0.6
6	ambroxide	2087	0.8	0.7	0.8	0.8
27	sesquiterpene alcohol (c)	2108	6.0	3.3	2.7	4.0
28	sclareoloxide	2152	0.3	0.3	1.1	1.3
29	15-nor-labdan-8-ol **	2295	1.7	1.8	5.2	3.5
	TOTAL	-	52.6	53.0	39.3	44.2

* Linear retention indices determined in a CP-Wax 52 CB column

** Tentatively identified. m/z(rel.int.): 280 (M+) (7), 262 (20), 247 (35), 222 (10), 204 (10), 191 (43), 177 (64), 163 (9), 153 (22), 137 (35), 123 (43), 109 (64), 95 (68), 81 (54), 69 (64), 55 (55), 41 (100).

(a) m/z(rel.int.): 137 (M+) (2), 119 (6), 105 (3), 94 (100), 93 (43), 91 (56), 79 (97), 77 (42), 65 (18), 59 (95), 51 (16), 43 (37), 39 (39).

(b) m/z(rel.int.): 264 (M+) (1), 222 (16), 204 (75), 189 (25), 179 (13), 162 (23), 161 (100), 150 (12), 133 (12), 119 (26), 105 (33), 95 (21), 79 (17), 67 (15), 55 (11), 43 (65), 39 (29).

(c) m/z(rel.int.): 222 (M+) (3), 204 (34), 189 (22), 179 (13), 161 (100), 147 (10), 133 (16), 119 (33), 109 (50), 95 (46), 81 (30), 67 (32), 55 (22), 41 (55).

Influence of growing site

Considering the oil composition of cistus plants from different sites, there were found some differences. The cistus oil of site 2 had a high content on the ocimenone isomers, an absence of trans-pinocarveol and unknown (component 17) and a higher quantity of less volatile components such as sclareol oxide and 15-nor-labdan-8-ol. Cistus oil from site 1 was richer in sesquiterpene alcohols and 2,2,6-trimethylcyclohexanone. The amount of ambrox was the same for both oils.

Considering the use of fresh or dry plant, the composition of cistus from site 2 was more affected, decreasing the amount of components of middle to high volatility and increasing the amount of the less volatiles. Drying promoted the doubling of the amount of ocimenone isomers in cistus oil from site 2 and of unknown (component 21) and sesquiperpene alcohol (component 29) in cistus from site 1. Again the quantity of ambrox was the same for both oils.

Organoleptic caracteristics

The cistus oil obtained in this work was transparent light-yellow coloured, with an initial fresh terpenic resinous pungent odour, causing great impact, and rapidly evolving to a strong sweeter, almost animal sweat, long-lasting scent.

From the olfactory point of view, besides the components already mentioned, there are other contributions to cistus oil odour, namely: viridiflorol (floral); terpinen-4-ol (spicy floral); bornyl acetate, borneol and myrtenol (fresh camphoraceous); (Z)-ocimenone (green herbaceous).

In conclusion, Portuguese cistus oil herein characterized is richer in amber-like components and has a low content of hydrocarbon monoterpenes, which is desirable from the organoleptic point of view.

Comparison with commercial samples

Commercial samples of cistus oils from Spain were also analyzed by GC/MS and compared with the essential oil obtained in this work. The chromatograms and the composition are shown in Figure 3.20 and Table 3.7, respectively.

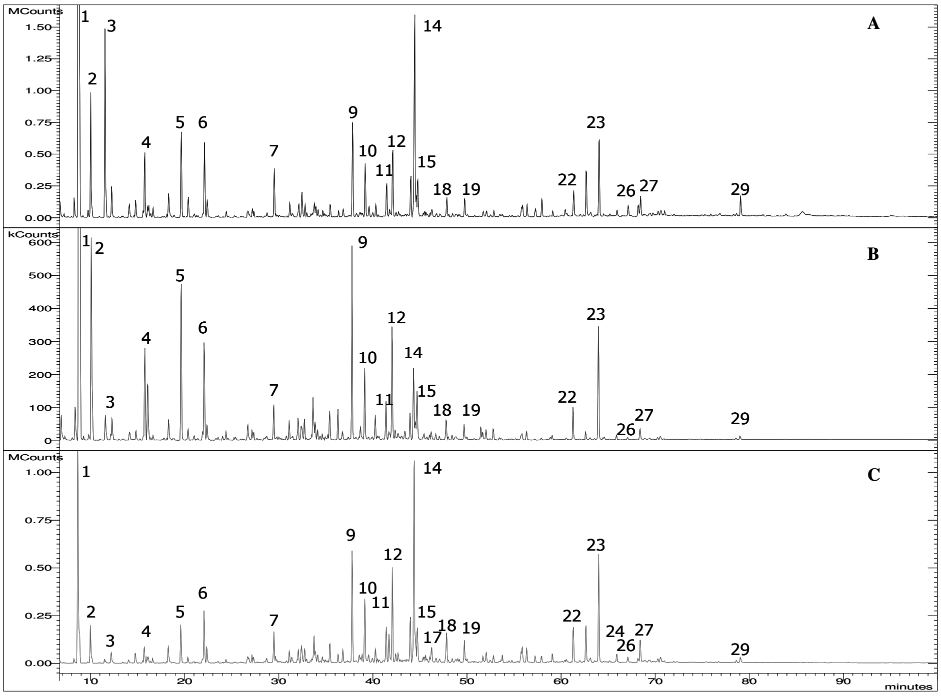


Figure 3.20 GC/MS chromatograms of commercial cistus oil: sample A, sample B and sample C (see table 3.7 for correspondence number-component).

Table 3.7 Percentage composition of commercial cistus essential oils produced in Spain.

N	Component	Sample A	Sample B	Sample C
1	α-pinene	50.4	48.9	12.8
2	camphene	2.4	5.0	1.9
3	β-pinene	4.4	0.6	0.2
4	limonene	1.4	2.3	0.8
5	p-cymene	1.9	3.6	1.9
6	2,2,6-trimethylcyclohexanone	1.5	2.0	2.4
7	p-cymenene	1.3	0.9	1.6
8	pinocarvone	0.2	-	0.8
9	bornyl acetate	1.5	3.1	3.5
10	terpinen-4-ol	0.9	1.4	2.5
11	allo-aromadendrene	0.5	0.6	1.2
12	trans-pinocarveol	1.7	2.8	4.9
13	(Z)-ocimenone	0.1	0.0	0.3
14	(E)-ocimenone	-	-	-
15	viridiflorene	3.7	1.4	8.0
16	borneol	0.7	1.1	1.6
17	unknown (a)	0.1	-	0.6
18	δ-cadinene	0.3	0.3	0.9
19	myrtenol	0.5	0.4	1.2
20	caryophyllene oxide	-	-	0.1
21	$C_{15}H_{26}O$ sesquiterpene alcohol (b)	-	-	0.2
22	globulol	0.4	0.5	1.3
23	viridiflorol	1.1	1.7	3.4
24	d-verbenone	0.3	0.2	0.7
25	(-)- spathulenol	0.1	0.1	0.3
26	ambroxide	0.2	0.1	0.3
27	sesquiterpene alcohol (c)	0.3	0.2	0.8
28	sclareoloxide	-	-	0.2
29	15-nor-labdan-8-ol **	0.2	0.1	0.3
	TOTAL	76.3	77.1	54.7

Samples A and B showed a very similar composition, with α-pinene constituting almost 50% of the oil (group A). On the other hand, sample C had a considerably lower content of this monoterpene (12.8%), possessing higher contents of the other components, namely 2,2,6-trimethylcyclohexanone, ambrox and 15-nor-labdan-8-ol, resembling more the Portuguese cistus oils here studied. All the commercial oils possessed lower contents of ambrox and other low volatility components than our oil.

The commercial cistus oils have a high content of monoterpenes that degrade easily. To obtain a product with better quality for perfumery it is necessary further processing, such as deterpenation or re-distillation. The fact that our oil has already low contents of monoterpenes constitutes an advantage for using the Portuguese plants for the production of good quality cistus oil.

3.3.6 Conclusions

The chemical composition of the cistus oil from Portuguese-grown rockrose (*Cistus ladanifer*) was determined. A comparison was also made in terms of geographical source and using either dry or fresh plant. The cistus oil herein obtained is richer in amber-like components and has a low content of monoterpenes when compared to the usual values found in the literature. Moreover it presented a relatively high content of ambrox (amber odour), which usually is only reported in labdanum oil. The major constituents were three sesquiterpene alcohols viridiflorol (13.6-17.4%), globulol (3.1-5.0%) and a unknown sesquiterpene alcohol (2.7-6.0%), as well as the diterpene alcohol 15-nor-labdan-8-ol (1.7-5.2%). Three samples of commercially available cistus oil were also analyzed and compared, having a high content of monoterpenes.

3.4 References

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CHAPTER 4

PRODUCTION OF ROSE GERANIUM OIL USING SUPERCRITICAL FLUID EXTRACTION

The present work aims to apply the supercritical fluid extraction (SFE) with carbon dioxide as a clean technology to extract the natural essential oil from a Portuguese-grown plant, the rose geranium (*Pelargonium sp*). The applications and the motivation for studying this aromatic plant have been already presented in Chapter 3, namely that geranium oil constitutes an important ingredient in perfumery as middle note and is a natural resource with potential to be explored in Portugal, since this plant is well adapted to our climate and soil.

In this chapter, a new SFE equipment was designed and constructed and it was tested by using it to the extraction of geranium extract. The composition of the obtained rose geranium extracts was determined by GC/MS and their organoleptic quality (colour and odour) was evaluated and compared with the materials obtained by the traditional techniques - hydrodistillation and organic solvent extraction, presented in Chapter 3.

4.1 Introduction

The most known and old application of supercritical CO_2 extraction technology (SFE) is in decaffeination of coffee and tea. Nowadays, the main applications of supercritical technology are the extraction of clean natural extracts and the upgrading raw materials such as the removal of pesticides from medicinal plants, cholesterol from animal products and solvents from synthesis products (Gerard and May, 2002; Mukhopadhyay, 2000). SFE is also widely used for flavours and fragrances applications (Reverchon, 1997), which is an increasing tendency due to the increasing restrictions on organic solvent residue trace amounts. In fact, CO2 extracts have the status GRAS (Generally Recognized As Safe) to be used in food products (Gerard and May, 2002). The increased cost of energy and demand for high quality products constitute also driving forces for the use of SFE.

4.1.1 Fundamentals of supercritical technology

Supercritical fluid extraction (SFE) uses fluids at pressure and temperature conditions that are above the critical point, having specific properties that are different from the liquid or gas phase – supercritical fluid region (Figure 4.1). In fact, the supercritical fluids combine the properties of gases and liquids in an advantageous way: they present comparable solvent power (density) to liquids but have a diffusivity considerably higher and a lower viscosity than liquids, which give them an increased ability to penetrate into the matrices and extract the solutes. Moreover, the dielectric constant also changes at supercritical conditions, so carbon dioxide that is non-polar at normal conditions presents a dielectric constant equivalent to polar solvents at high pressures and temperatures, while water becomes non-polar with a value close to zero (Lanças, 2000).

The composition of the natural material is different according to the extraction method employed. At this point, it is important to make the following distinction: essential oil is obtained by distillation only and is completely volatile. The remaining products obtained from natural sources by extraction with solvents and SFE are extracts and contain a volatile fraction (which may contain the whole essential oil

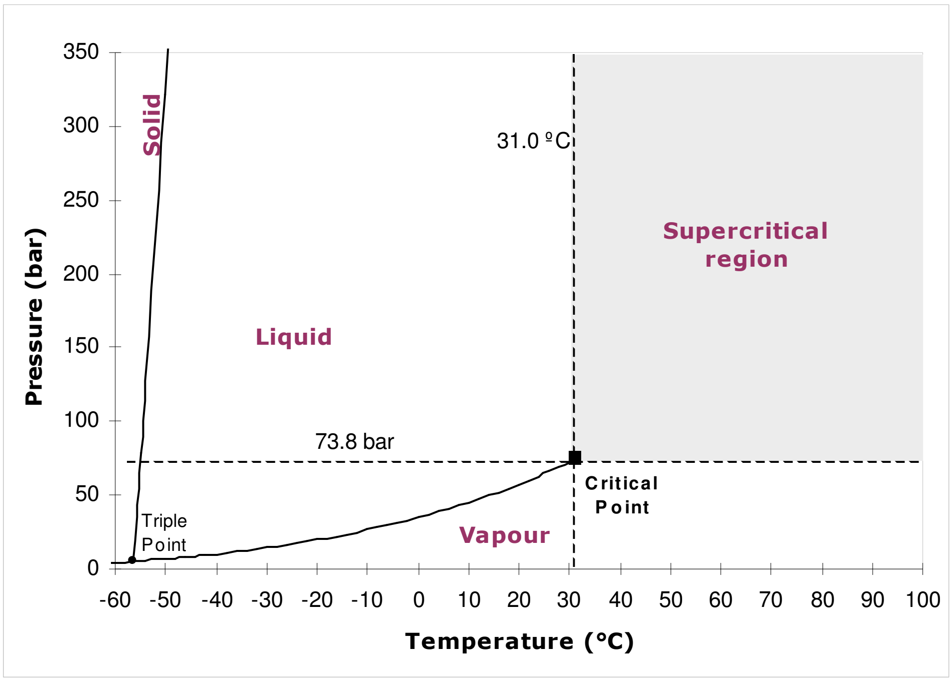


Figure 4.1 Phase diagram of carbon dioxide.

or just part of its composition) and other non-volatile materials like waxes and pigments. It is desirable that the extracts contain as much as possible of the essential oil existing in the plant material and that the co-extraction of non-volatiles is minimized.

Figure 4.2 illustrates the different composition profiles of naturals obtained by different processes. This representation is like a GC chromatogram, where the x-axis is the retention time and the y-axis is the amount of component. The lower the retention time, the higher is the volatility of the component (Brogle, 1982). So, the essential oil is the most volatile fraction, while resins and pigments are the high-molecular weight and non-volatile components. Comparing the profiles, it can be seen that the steam-distillation product comprises only the volatile essential oil fraction and other volatile components formed during the distillation process. On the other hand, the extraction with non-polar solvent corresponds to the total extract, comprising almost all range of components except for the most volatile components, partly lost during solvent recovery from the extract. Using instead a polar solvent, the extraction is more selective but still extracts non-volatile polar constituents such as sugars, tannins, salts, and so on. The loss of volatiles is also more severe. Finally, the SFE with CO_2 as solvent,

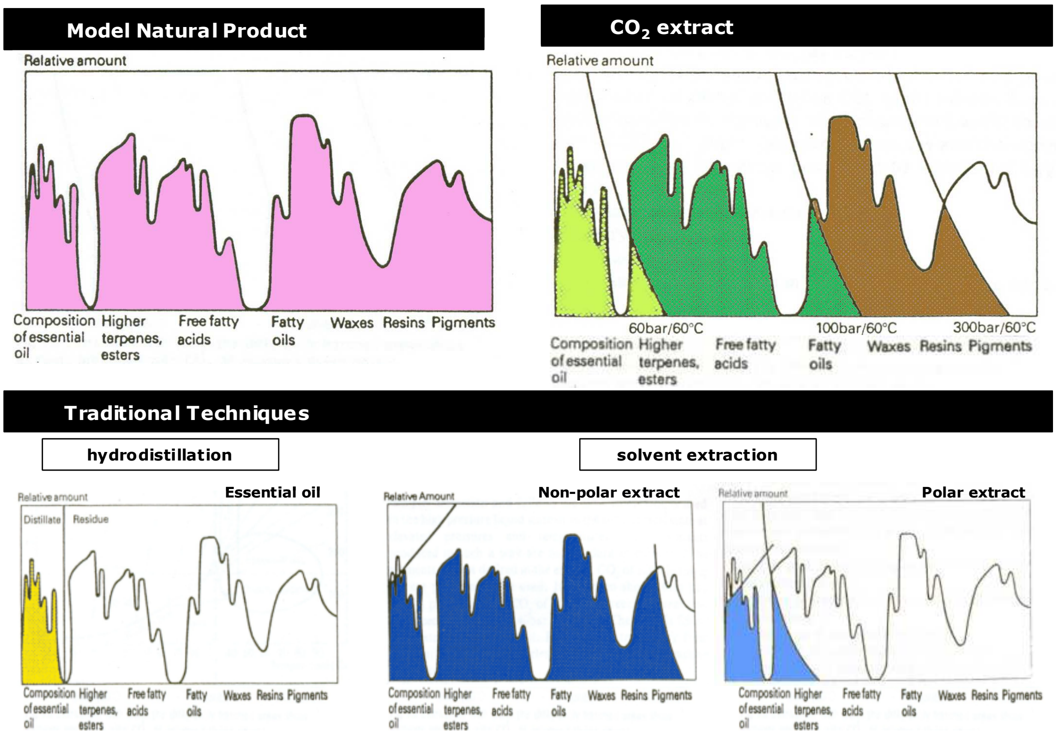


Figure 4.2 Composition profile of the natural material and the corresponding products obtained by different methods: coloured area - composition of the extract, white area – composition of residue or losses (adapted from Brogle, 1982)

yields different extract profiles according to the operating conditions used and there is negligible loss of volatiles. To get a profile as close as possible to the steam-distilled essential oil, CO_2 must be used at a low solvent power, i.e., near the critical point.

The traditional extraction methods present many drawbacks. The distillation process uses relatively high temperatures during a long time, leading to the decomposition of the flavour and fragrance molecules by heat, hydrolysis, isomerization, polymerisation of aldehydes and oxidation, which many times results in the undesired formation of off-flavours or off-odours. On the other hand, extraction with organic volatile solvents has a low selectivity, co-extracting non-volatiles pigments and waxes together with the essential oil, and so the extracts are usually dark coloured and viscous, rendering them difficult to handle. There are also environmental and health problems related to the handling of large quantities of hazardous solvents, the energy-intensive processes for concentrating the extract and reusing the solvent, the contamination with solvent residues (that may be toxic, reactive or influence the organoleptic characteristics) and the loss of volatiles when the solvent is removed. Taking all this in consideration, it is very encouraged the development of new methods of extraction that provide aromatic extracts of superior quality, as is the case of SFE technology.

SFE is a versatile technology that enables the production of tailor-made flavour and fragrance, with a specified profile (Gerard and May, 2002). As SFE extraction uses gentle operating conditions (low temperature, close to ambient), the SFE extract has been recognized as having a superior quality in terms of odour and taste, with fresher characteristics and resembling more to its natural source, especially when compared with distilled oils.

The conditions in the CO_2 critical point, CP, are: critical temperature, 31.04°C and critical pressure, 73.04 bar. The selectivity can be adjusted by setting the operation conditions of pressure and temperature, as is shown in Figure 4.3: at low pressures close to the critical point, CP, the volatile essential oil is selectively extracted, while at high pressures the total extract is obtained, including the non-volatile lipophilic components such as waxes, pigments and bioactive constituents. The extract can be further refined by fractionation either by making stepwise separation or by using column chromatography also at supercritical conditions.

One important advantage of SFE extraction is that more stable extracts are obtained, which are naturally preserved because the micro-organisms and spores do not resist to the violent pressure gradient that occurs in the end of SFE depressurisation process to recover the extract (Gerard and May, 2002).

Other advantages of SFE include:

- the solvent power is tuned by mechanical pressure and/or temperature;
- can be applied at different scales, from analytical (less than 1g) to industrial scale (several tons);
- oxygen free operation, which prevents oxidation reactions during extraction;
- the solvent is gas at normal conditions so it is easily removed by releasing the pressure and so the extract obtained is completely solvent-free, eliminating the solute concentration (or solvent recovery) step;
- render faster extractions (low viscosities, high diffusivities, good solvating power), continuous, with better energetic efficiency (lower temperatures and no solvent recovery).

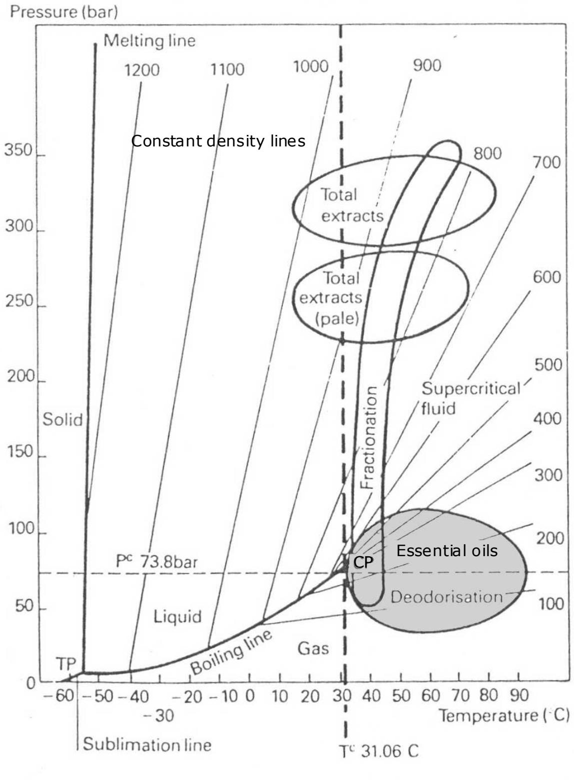
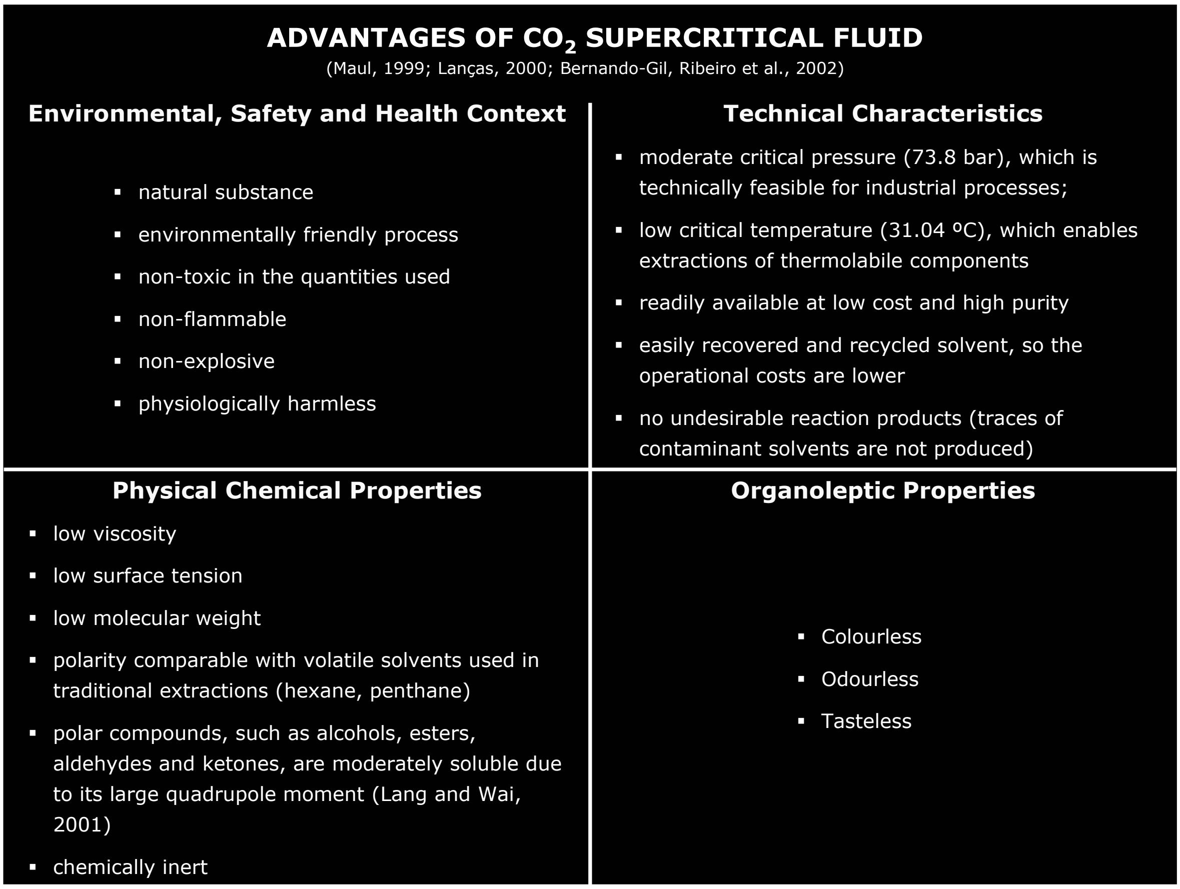


Figure 4.3 The various natural extracts obtained by SFE, using different conditions of pressure and temperature (adapted from Brogle, 1982)

The main advantages of using CO_2 as supercritical fluid are shown in Figure 4.4.

The main drawback of SFE technology is the high pressures required, which implies higher investment costs and safety precautions as the provision of high pressure relief valves, vent discharge lines and adequate ventilation to prevent asphyxiation risk by high concentration of CO_2 (Imison and Unthank, 2000). From the technical point of view, there are also problems with extraction/fractionation rate, solubility and selectivity, being necessary a pre-treatment of the plant material (milling under cryogenic conditions, for example) and there is a tendency to co-extract undesirable constituents, such as waxes. The high pressures also make the continuous introduction of solids difficult and imply high operation costs. For all these reasons, SFE is profitable only when applied to high-added value (Bernando-Gil *et al.*, 2002), to obtain ultra-pure products or if imposed by regulatory restrictions on residues.



ADVANTAGES OF CO_2 SUPERCRITICAL FLUID

(Maul, 1999; Lanças, 2000; Bernando-Gil, Ribeiro et al., 2002)

Environmental, Safety and Health Context

- natural substance
- environmentally friendly process
- non-toxic in the quantities used
- non-flammable
- non-explosive
- physiologically harmless

Technical Characteristics

- moderate critical pressure (73.8 bar), which is technically feasible for industrial processes;
- low critical temperature (31.04 ºC), which enables extractions of thermolabile components
- readily available at low cost and high purity
- easily recovered and recycled solvent, so the operational costs are lower
- no undesirable reaction products (traces of contaminant solvents are not produced)

Physical Chemical Properties

- low viscosity
- low surface tension
- low molecular weight
- polarity comparable with volatile solvents used in traditional extractions (hexane, penthane)
- polar compounds, such as alcohols, esters, aldehydes and ketones, are moderately soluble due to its large quadrupole moment (Lang and Wai, 2001)
- chemically inert

Organoleptic Properties

- Colourless
- Odourless
- Tasteless

Figure 4.4 Main advantages in using carbon dioxide as supercritical fluid.

The non-polarity of supercritical CO_2 can be overcome by using modifiers (polar solvents, surfactants or other additive) to increase its affinity to more highly polar components. The most used solvents are hexane, ethanol, methanol and acetone (Bernando-Gil *et al.*, 2002). However, the process is more complex and the scaling-up is also more difficult, because there is the problem with the solvent and residues elimination, not compensating the increase in the solvent power. Moreover, it can result in poor selectivity, extracting more undesirable components.

The co-extraction of undesirable components can be minimised in two ways (Reverchon, 1997):

- by a careful selection of the extraction parameters in order to avoid the extraction of waxes and other non-volatiles; in this case, the extraction should be carried out at pressures below 100 bar and at temperatures around 40-50 °C;
- extract at high pressures to have higher yields, and then promote a fractionated separation, where the waxes are precipitated in one separator vessel and the extract rich on essential oil on the other.

4.1.2 Description of plant material and its processing before extraction

In the extraction of plants, the diffusion of the volatile components out of the vegetable matrix is usually the limiting step, being influenced by three factors (Lang and Wai, 2001): (1) the entrainment of CO_2 molecules into the matrix, which would reduce the affinity of solute and plant material; (2) the solubility of solutes in supercritical CO_2, which is related to fluid density and solute volatility; and (3) the volatility of the solutes, dependent on the extraction temperature, especially those with high boiling points. The solubility factor is the most determinant for extraction efficiency.

To improve the extraction and yield of the volatiles from the plant material, there is a previous preparation step before SFE. The usual pre-treatments consist of milling or grinding the plant, which results in losses of volatile material by evaporation and eventually losses by degradation (by oxidation, heat or light exposure). The use of cryogenic conditions, the losses of volatile material and degradation are minimised.

The plant raw materials are usually used dried or mixed with a drying agent such as anhydrous sodium sulphate or silica-gel, because the moisture may cause clogging problems due to ice formation when the temperature drops during expansion of supercritical fluid, (Lang and Wai, 2001). Heating the restrictor or expansion valve can prevent these clogging problems and deposition of extracted components in the tubing or restrictor. In some cases, it could work as a modifier to increase solubility of components. There are several drying methods, namely freeze-drying (providing highest quantity of volatile components), oven-drying at 45 °C, vacuum rotary evaporation at 35 °C and at room temperature, in a ventilated place.

4.1.3 The supercritical extraction process

The supercritical fluid extraction (SFE) comprises four main steps, namely:

(i) charging of extractor with plant material;

(ii) compression and heating of CO2 to the extraction pressure and temperature, respectively;

(iii) extraction of volatiles or other material of interest (antioxidant, pharmaceutical active compounds, etc);

(iv) separation of extract from CO2 and recovery of the extract, usually by stepwise depressurization and cooling, where CO2 passes to the gas phase, the extract precipitates as a pure product, completely free of solvents.

There may be also a **(v)** stage of recycling the CO2 used.

SFE can be carried out in static or dynamic mode. In the static mode, the supercritical CO2 is contacted with the vegetable matrix for some time in order to extract the volatile fraction by dissolving it and the separation/recovery of the SFE extract occurs only in the end. This is a batch mode operation. The extraction yield is limited to the equilibrium concentration of volatile fraction in the supercritical CO2, at the extraction conditions of pressure and temperature. In the dynamic mode, a continuous flow of supercritical CO_2 go through a bed of plant material placed inside the extractor, the volatiles dissolve in the fluid and is immediately carried away to be continuously recovered in the separator. SFE in the dynamic mode is a semi-continuous process in the case of extraction of plants (the extractor must be charged with plant raw material, from time to time), and can be entirely continuous for fractionation of liquid streams, such as deterpenation of citric oils. In some cases, the static and dynamic modes are combined, starting with static mode to stabilize and allow a better entrainment of CO2 in the vegetable matrix, and only afterwards starts with the dynamic mode.

The selection of the extraction variables can be adjusted to obtain extracts with specific organoleptic (fragrant) characteristics, as has been referred previously.

The physical properties of the solute, namely vapour pressure, polarity and molecular weight, affect its solubility in the supercritical fluid (Temelli *et al.*, 1988). The terpene hydrocarbons are non-polar, have lower molecular mass and a higher vapour pressure, so they have a high solubility in carbon dioxide than oxygenated components. However, all the components with molecular mass below 250, as is the case of most flavour and fragrance substances, are considerably soluble in supercritical CO_2 (Mukhopadhyay, 2000).

The SFE yield, solubility, extraction time and selectivity can be optimised by adjusting the density by changing the pressure and temperature conditions. In fact, the extraction is highly sensitive to these two parameters in the vicinity of the critical point. Other operational parameters influencing the yield and rate of extraction are the pre-treatment of plant material (drying, milling, etc), solvent flow rate, extraction time and design of extractor (relation height/width). The favourable transport properties promote deeper entrainment of solvent into the vegetable matrix and so faster extraction. The extraction time is important when the components have very different diffusion rates, so extracts obtained at different extraction times will have different composition (Reverchon, 1997).

Considering the effect of temperature, the vapour pressure of components to extract increase as temperature increases. Conversely, the density of supercritical CO_2 decreases and so does its solvating power. The effect of temperature changes with the range of extraction pressure: near the critical point, the decreasing of fluid density with the rise of temperature is the dominant effect and so the solubility diminishes; however, at high pressures, the increase of vapour pressure of the components to extract as the temperature rises overcomes the decrease of density, and so the solubility increases (Temelli *et al.*, 1988).

In general, at a given temperature, the solubility increases by increasing the pressure, as the density and its solvating power also increase. However, the selectivity and diffusivity diminishes, which affect the rate of extraction.

The flow rate of CO_2 determines if the extraction rate is solubility-controlled or diffusivity-controlled (Imison and Unthank, 2000). On the other hand, the operating costs and yield increase for higher flow rates.

The optimisation of processing conditions is therefore essential to meet competitive and product requirements.

Examples of SFE operating conditions applied to plants for fragrance extraction are presented in Table 4.1.

4.1.4 Description of separation methods

In the end of a supercritical fluid extraction process, the extract must be separated from the solvent CO2. The simplest separation (recovery) of the solute from the supercritical solvent consists of releasing the pressure to room pressure in a collection vessel at a low temperature (Akgun *et al.*, 2000; Einaki *et al.*, 1999; Machado *et al.*, 1993; Sargenti and Lanças, 1994).

Nevertheless, the separation or collection methods of supercritical extract that are described in the literature are done most commonly in two-stages: in separator 1, at lower temperature than extraction and at an intermediary pressure between extraction and ambient pressure, where the waxes and other undesired material are precipitated; and separator 2, which usually is at atmospheric pressure and low temperatures (–20 to 0 ºC), where the volatile fraction is retained.

Table 4.1 Operating conditions of carbon dioxide supercritical fluid extraction to plants for application in perfumes.

Natural raw material	**P (bar)**	**T (ºC)**	**CO_2 consumption (kg/h)**	**Application**	**Reference**
Portuguese – grown rose geranium leaves	160	36.5	-	Headspace analysis	(Machado *et al.*, 1993)
Spanish-grown Rockrose leaves	90	40	0.7	Fractionation at separation: S1-70 bar, -10 ºC; S2 –20 bar, -10 ºC	(Rincón *et al.*, 2000)
Portuguese-grown aromatic plants (rosemary, thymus, origanum)	70-180	40	0.06-0.2	Fractionation at separation: S1-10 bar, room temperature; S2 –1 bar, -60 ºC; (P1-P2<40 bar)	(Ferreira *et al.*, 1998; Moldão-Martins *et al.*, 1998; Nolasco *et al.*, 1998; Rodrigues *et al.*, 1998)
Flower concretes (orange flower, jasmine, rose, tuberose)	80-90	40-50	0.55-4.5	Fractionation at separation: S1-80/90 bar, -16/-10 ºC; S2 –15/25 bar, -10/10 ºC	(Reverchon and Marrone, 1997; Reverchon and Poletto, 1996; Reverchon and Porta, 1996; 1997; Reverchon *et al.*, 1995; 1997; Reverchon *et al.*, 1999)
Vanilla	100-130	33-36	0.001-12	-	(Nguyen *et al.*, 1991)
Peppermint (Mentha piperita)	65-100	25-40	-	-	(Ammann *et al.*, 1999)

The decrease of temperature is used in combination or as an alternative to precipitate and separate the extract - cryogenic trap. This is particularly useful when keeping the pressure relatively high when recycling the solvent, saving on the pressurization costs.

The separation vessel, especially at pilot and industrial scale, is usually empty and the extract precipitates on the walls. To facilitate the collection of extract the vessels are cyclones. At analytical scale, as involves the handling of small quantities of extract, the separator is filled with organic solvent to retain the solute – solvent trap (Rozzi and Singh, 2002; Turner *et al.*, 2002). The trapping solvents may be hexane, methanol, dichloromethane, acetone, ethanol or ethyl acetate. This is not applied to industrial scale since there is the obvious disadvantage of contaminating the extract with trace residues. Alternatively, a solid-phase extraction packing (SPE) or a cryogenic capillary may be used (Turner *et al.*, 2002).

4.2 Design and construction of a SFE equipment

The supercritical fluid extraction SFE set-up used was completely designed and built from the start during this work. The equipment was named **S**CRITICAL, and its layout is shown in Figure 4.5.

The SFE set-up is divided in four areas in terms of operating pressure: (1) medium pressure area, around 60 bar, where the CO2 is fed to the system and cooled before entering the pump; (2) high pressure area, with working pressure up to 200 bar, where CO2 is compressed and heated to supercritical conditions and the supercritical extraction is performed; (3) medium pressure area, with pressures in the range 20 to 50 bar, corresponding to the first depressurization step made trough regulation valve, VR1; and (4) the low pressure area, where the second depressurization stage occurs through a second regulation valve, VR2, and the extract is separated (recovered) from CO2 stream. It is possible to perform fractionation of the SFE extract by placing a separator in area (3). It is also possible to use an auxiliary pump to feed a modifier stream in area (1).

The pressurization of the system is accomplished by a high-pressure pump and a backpressure regulator: the pump works until the aimed pressure is reached, with valve V2 or V3 closed; if the set pressure is exceeded, the backpressure opens and releases the pressure in the system. The pressure is

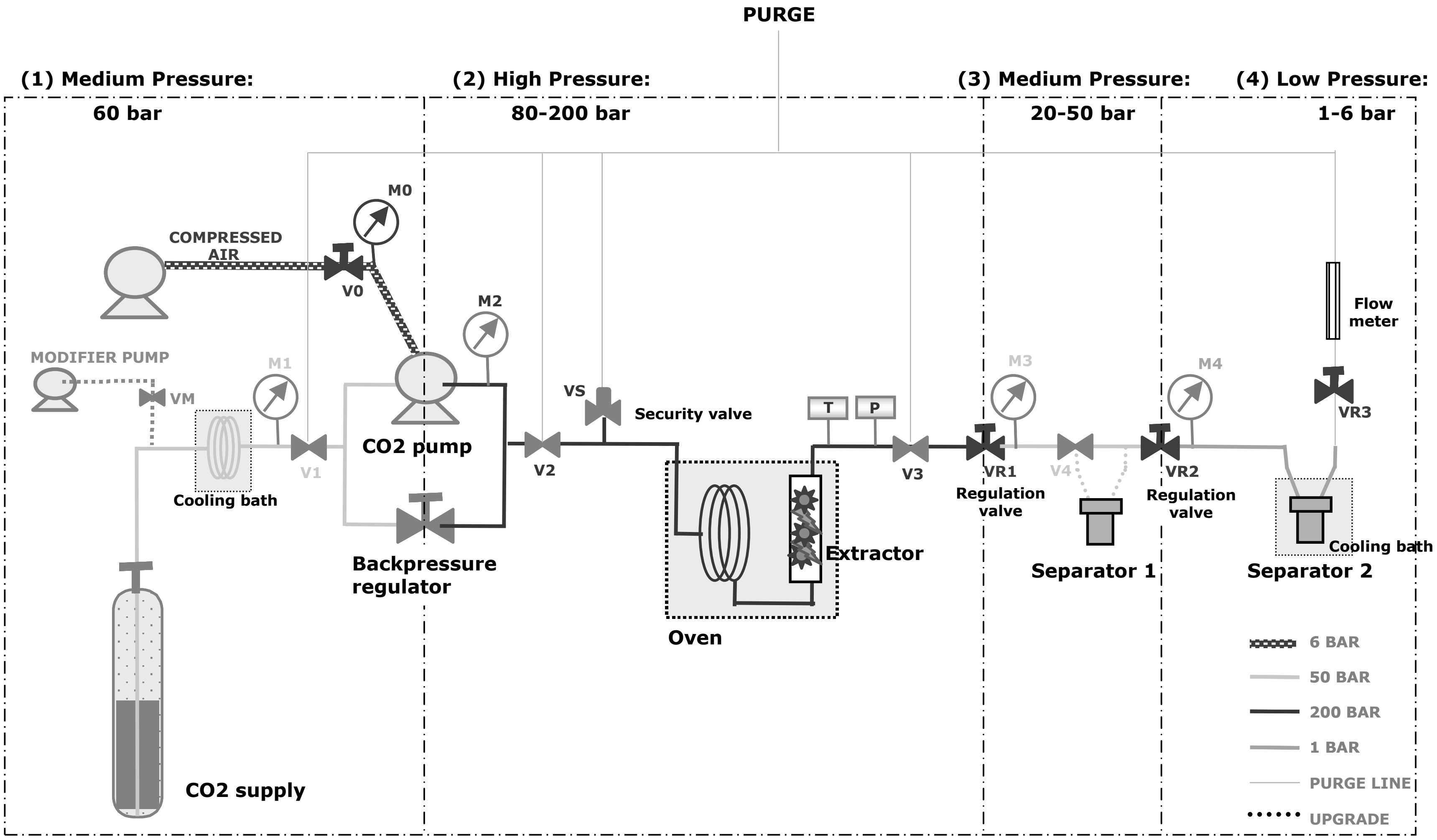


Figure 4.1 . Schematic representation of supercritical fluid extraction (SFE) set-up

monitored by manometer M2 and adjusted by controlling simultaneously the pump flow (by setting the pressure of compressed air using valve V0) and the backpressure regulator opening. This configuration in parallel enables to maintain the high pressure in the system, between valves V2 and V3. The CO2pump head and CO2 stream before entering the pump are cooled in order to prevent cavitation inside the pump.

For security reasons, since operating at high pressures, there is a security valve, VS, regulated to release the pressure if it exceeds 190 bar, leading CO2 stream to a purge line.

The extractor is placed inside an oven, after charged with plant and closed. The extraction can be performed in static or dynamic mode. In the static mode, valve V3 is closed, and the plant and Co2 are left in contact for a period of time. In dynamic mode, valve V3 is open and CO2 flows continuously through the bed of plant. The extraction pressure and temperature are monitored by a pressure transducer (P) and a thermocouple (T), respectively.

The depressurization step is performed using two regulation valves, VR1 and VR2. The pressure in each step is monitored by manometers M3 and M4. The valves VR1 and VR2 are heated by a heating resistance strip controlled by a thermostat, in order to compensate the intense cooling due to Joule-Thomson effect and to avoid clogging of valve and tubing with ice.

The collection of extract may be performed at medium pressure between regulation valves VR1 and VR2, in Separator 1, and at low pressure after VR2, in Separator 2. The set-up is easily adjustable to add more separators, by placing more valves and/or more separators in the system. The Separator 2 is placed inside a cooling bath (water with salt) because lowering the temperature, is minimized the loss of volatile fraction of extract carried away with gas CO2 stream.

In the end, there is a third regulation valve VR3 to control CO2 flow rate, which is measured by a gas flow meter, at atmospheric pressure.

4.2.1 Description of SCRITICAL equipment

The SFE equipment is designed to work up to 200 bar, an appropriate pressure range for most of the extractions of flavour and fragrance materials from plants.

The supercritical extraction equipment is built in stainless steel material, within a box with dimensions of 65×35×35 cm. The control of the extraction process is made through a control panel, where all the valves and manometers are mounted, as can be seen in Figure 4.6. The security valve, the extractor and the separators are outside **S**CRITICAL, being the connections between these equipments made through the rear panel. The front and rear panels are supported in the box. Inside this box it is placed the high-pressure pump and all the connections between the valves, back-pressure regulator, pump, manometers, flow meter, etc.

The CO_2 (Praxair, Portugal) is supplied liquid in cylinders with dip tubes at 60 bar from Praxair, with a purity of 99.9% (food industry grade). The CO_2 supply line is around 60 bar (pressure of the CO_2 bottle) and is cooled in a stainless steel coil immersed in an ice bath before going into the pump, in order to ensure that CO_2 is in the liquid phase.

The high-pressure pump (model M71, Haskel, USA) is air-driven piston type pump, requiring only compressed air to be operated. The flow-rate is set by the pressure of compressed air, so that increasing the air pressure, increases the number of strokes/min and the flow-rate is increased. This pump model has the capacity of multiplying the air pressure 71 times, which means that for setting the SCRITICAL at a pressure of 100 bar, the compressed air pressure must be 1.4 bar. If the system is closed (static mode, valve V3 closed), then the pump stops working when the set pressure is reached. The advantages of using this kind of pump is its high pumping capacity and being able to reach high pressures rather fast. The main disadvantage is that the movement of the piston leads to heating by friction after long operating times and the piston can be damaged. Moreover, the system is considerably noisy.

The backpressure regulator, BP (Tescom, USA), is manually regulated, by turning a knob (see Figure 4.6).

The oven (air bath) has a temperature controller, which enables to use a temperature programme along the SFE extraction. There is also circulating air, to homogenize the temperature inside the oven. The internal volume of the oven is 150 liters. The supercritical CO_2 is heated before entering the extractor by going through a stainless steel coil placed inside the oven. The tubing connecting the outlet of extractor and regulation valve VR1 is isolated with glass wool to minimise heat losses and so prevent condensation of extract on the tubing. Figure 4.7 shows the oven used in this set-up.

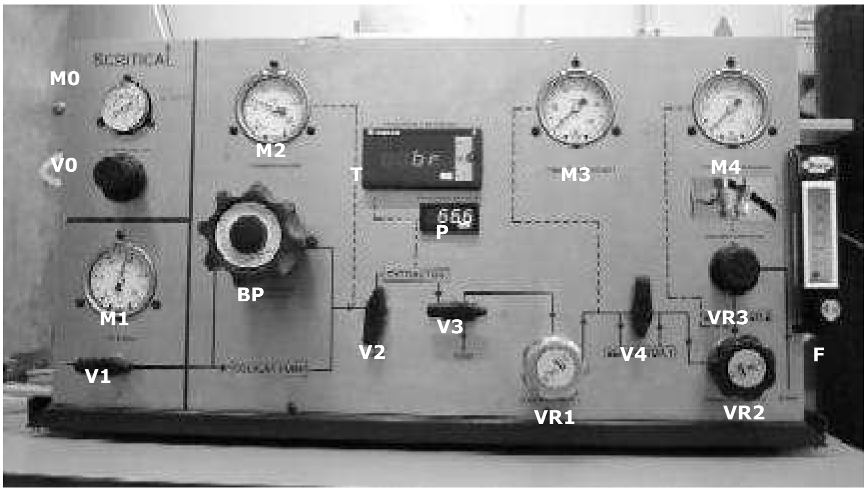


Figure 4.6 Supercritical extraction equipment, front panel of SCRITICAL :V-ball valve; VR–regulation valves; BP-backpressure regulator; M-manometers; F-flow-meter; P-extractor pressure reading; T-extractor temperature reading.

Figure 4.7 Supercritical extraction equipment, oven.

The extractor is made of stainless steel material. The extractor was built with a cylindrical shape, with dimensions 6.2 cm I.D. and 35.1 cm height, having the capacity of approximately 1 L (see Figure 4.8). The extractor stands vertical using a tripod support. At the top cover of the extractor there is a filter to prevent down clogging of the tubing with plant material or residues. Inside the extractor it is placed a basket to hold the plant material and its base works as a fluid distributor (see Figure 4.9).

As is was described in section 4.1, the depressurization is done in two stages using the regulation valves VR1 and VR2 (Veriflo, USA), first to an intermediary pressure of 20 to 50 bar and then to 1 to 6 bar. The regulation valves VR1 and VR2 are operated manually. These valves are heated, for the reasons already mentioned before.

In the end, CO_2 gas flow rate is measured by a flow meter, F (Dwyer, USA).

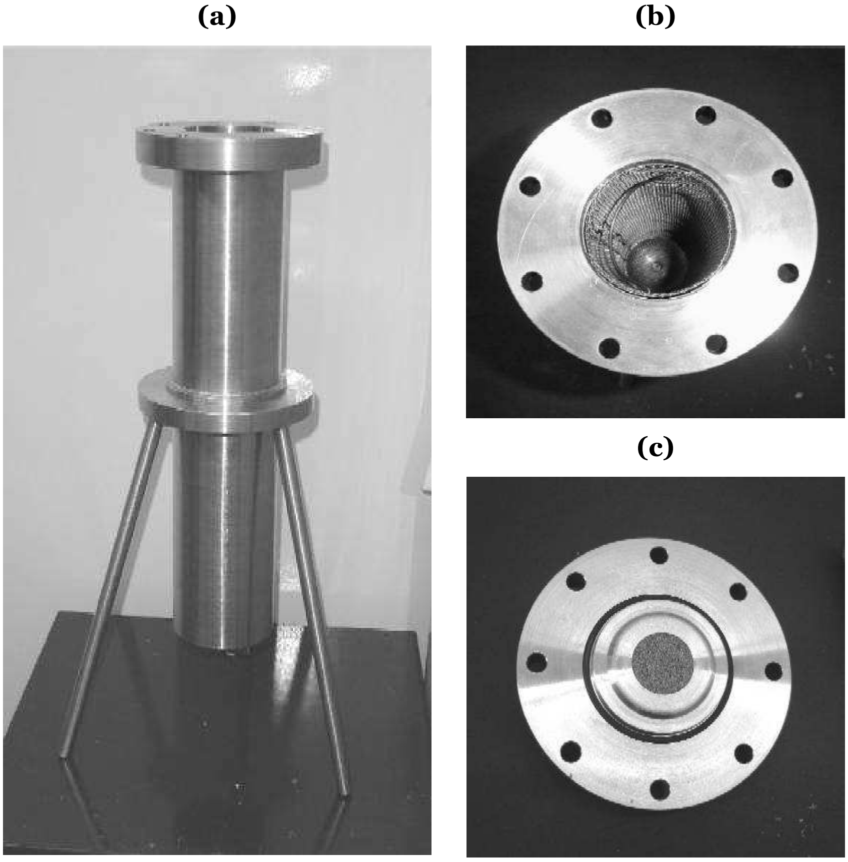


Figure 4.8 Supercritical extraction equipment, extractor vessel: (a) side view; (b) top view; (c) top cover.

Figure 4.9 Supercritical extraction equipment – plant basket.

4.3 Materials and methods

4.3.1 Plant material

The samples consisted of green leaves of rose-geranium plant *Pelargonium sp.*, air-dried overnight. The plant was collected in December/January, during winter season, from plants cultivated in the North of Portugal (humid climate). These plants were propagated by stem cuttings from a wild plant found in the South of Portugal (dry climate).

The leaves were cut into small pieces (Figure 4.10) of 5 to 20 mm and placed into the basket.

To study the effect of pre-treatment of the matrix, the plant was ground to small particle size of 1 to 5 mm (Figure 4.11). Before grinding, the plant was frozen in order to minimise the loss of volatiles.

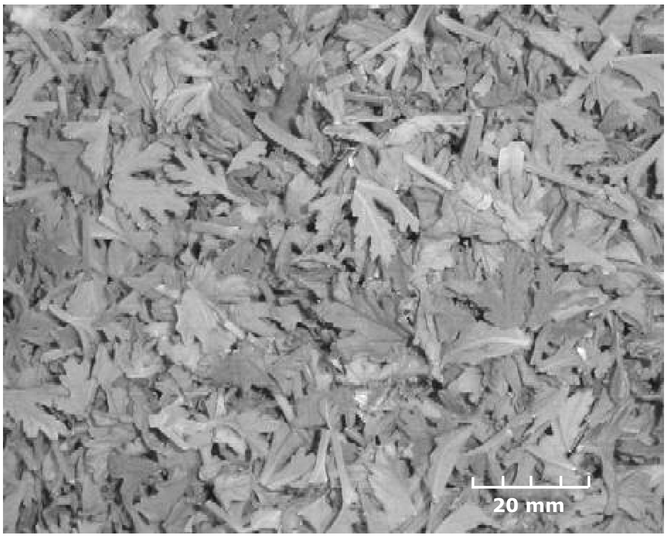


Figure 4.10 Rose geranium leaves cut for supercritical extraction.

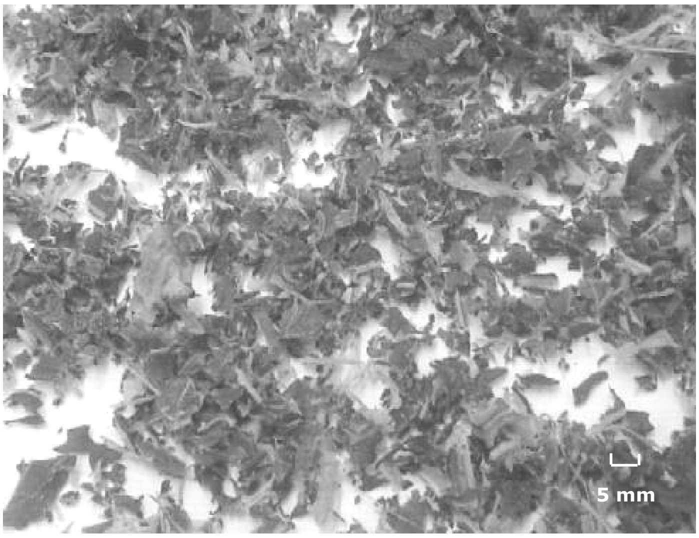


Figure 4.11 Rose geranium leaves ground for the study of effect of plant pre-treatment in supercritical extraction.

4.3.2 Methods

SFE extraction

The CO2 bottle was opened, keeping valve V2 closed, filling the pump with liquid CO2. The pump was turned on by increasing the air compressed pressure. The extraction pressure was set by regulating compressed air pressure (valve V0) and backpressure regulator, at the same time.

The basket with the plant was weighted and placed inside the extractor. The extractor was closed and placed inside the oven. After that, valve V2 was opened, keeping valve V3 closed, and the extractor was pressurized up to the extraction pressure. The stabilization of pressure and temperature took 18 to 20 min. In the course of the experiments, the temperature and pressure oscillation was ± 2 °C and ± 1 bar, respectively.

The experimental runs to study the influence of temperature, pressure and plant pre-treatment were carried out in the static mode, leaving supercritical CO2 in contact with plant material for 2 hours. The study of the influence of time of extraction was also carried out in the static mode, changing only the time of extraction. To study the influence of the operating mode, the experiment was run in dynamic mode, with valve V3 open and the pump continuously working, for 15 min, at a CO2 flow rate of 1.6 kg/h, with VR2 regulated so that the outlet pressure was 2 bar. After extraction time, valve V2 was closed and valve V3 was opened to depressurize the extractor. Regulation valve VR1 was regulated so that the outlet pressure was half of the inlet pressure down to 20 bar (maximum outlet pressure of 65 bar). Regulation valve VR2 was fixed at an outlet pressure of 5.5 bar.

The separation or extract recovery was made only through one separator placed after VR2, at low pressure and in a cooling bath of ice and salt (at -10 to –5 °C). As the quantities involved were small, the separator was filled with hexane and the gas stream passed through the solvent so that the extract was trapped in the hexane. After the experiment, the tubing and valves were washed with hexane to collect also the extract eventually condensed there. The extract was then concentrated to the concrete using a rotary evaporator under reduced pressure, with bath temperature of 45 °C. Afterwards the concrete was extracted with ethanol, heated to dissolve extract, cooled in the freezer to precipitate the waxes and then filtrated under vacuum and low temperature, to obtain finally the absolute.

The parameters studied are shown in Table 4.2. On the left column the constant operating conditions are shown, while on the right column the values of the variable parameter under study.

The experiments were performed only once (sometimes twice) due to the restriction of the small amount of plant available.

The essential oil of the plant used in SFE study was obtained by hydrodistillation as a control sample for the composition of the extracts, since it is known that natural materials suffer variations along the year.

Table 4.2 Parameters used in the study of supercritical extraction of rose geranium.

Reference conditions	**t=120 min** **T=60 °C** **P=100 bar** **cut plant (5-20 mm)** **static mode**
Constant parameters	**Varying parameter**
	Extraction time (t)
T=60 °C	5 min
P=100 bar	15 min
	30 min
	60 min
	180 min
	Temperature (T)
t=120 min	40 °C
P=100 bar	80 °C
	100 °C
	Pressure (P)
t=120 min	80 bar
T=60 °C	160 bar
	Plant pre-treatment
t=120 min	cut plant (5-20 mm)
T=60 °C	ground plant (1-5 mm)
P=100 bar	
	Operating mode
T=60 °C	static : 15 min
P=100 bar	dynamic: 15 min, 1.6 kg CO_2/h

Characterization of SFE extracts

The composition of SFE extracts was determined by GC/MS analysis of the obtained absolute. The methodology of quantification and identification of components was the same already described in Chapter 3 (sections 3.1.4.3 and 3.1.4.4). The extracts were also compared with the essential oil and solvent extracts that have been obtained by the traditional techniques of hydrodistillation and solvent extraction, characterized in Chapter 3.

4.4 Results

4.4.1 Comparison of SFE extract with products obtained using traditional techniques

The SFE extraction yield was calculated as the ratio of the mass of extract or essential oil obtained and the mass of the fresh plant used for extraction. The SFE extract considered for comparison was the one obtained at the reference operating conditions (60 °C, 100 bar, 120 min).

Figure 4.12 shows the range of extraction yields obtained by hydrodistillation (HD), solvent extraction with hexane (SE) and supercritical fluid extraction (SFE). It can be seen that the extraction yield was different for the three extraction methods considered. HD gives the lowest yield because this method delivers only the volatile fraction constituted of essential oil, while SE method shows the highest yield since a complete extract was obtained, including pigments and waxes. On the other hand, SFE showed an intermediary yield between the other two techniques, being more selective than SE but still having some co-extraction of non-volatiles.

The components identified and quantified in geranium oil and extracts are shown in chromatograms of Figure 4.13 and Table 4.3. Note that only the main components that are representative of the different kinds of the existing components are shown, such as hydrocarbon and oxygenated terpenes and sesquiterpenes, covering the whole range of volatility. The composition of essential oil (EO) obtained from the same plant used for SFE experiments was also included as a control sample of the composition, since there are always natural variations when dealing with plant materials. In fact, it

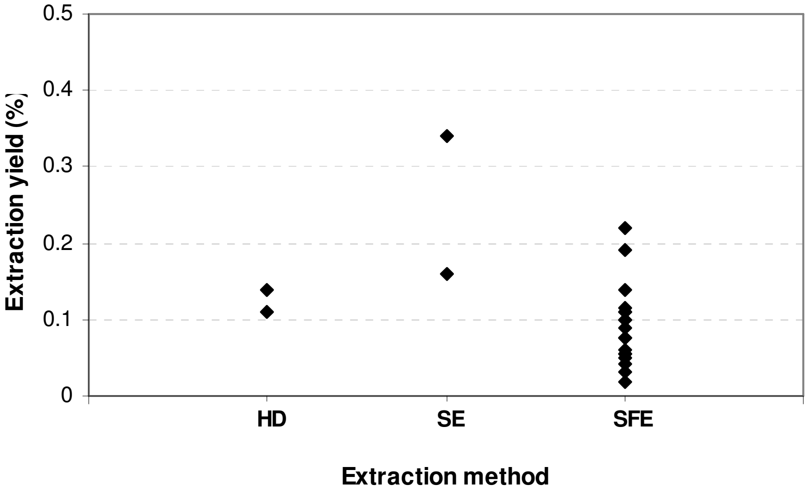


Figure 4.12 Extraction yields for Rose geranium, obtained by different methods: hydrodistillation (HD); solvent extraction with hexane (SE) and supercritical fluid extraction (SFE).

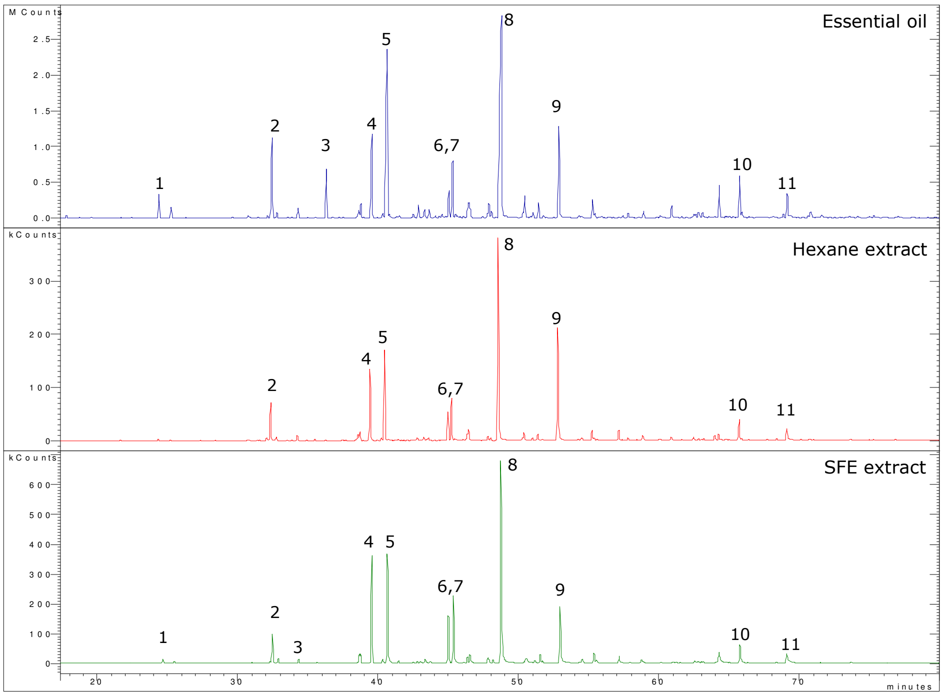


Figure 4.13 GC/MS chromatograms of geranium oil and extracts obtained with different extraction methods. 1. rose oxide; 2. isomenthone; 3. linalool; 4 guaia-6,9-diene; 5. citronellyl formate; 6. germacrene-D; 7. geranyl formate; 8. citronellol; 9. geraniol; 10. geranyl tiglate; 11. 2-phenylethyl tiglate.

Table 4.3 Percentage composition of geranium essential oil and extracts obtained with different extraction techniques.

		TRADITIONAL TECHNIQUES		**CONTROL SAMPLE**	**CLEAN TECHNOLOGY**
No.	**Component**	**Hydrodistillation**	**Solvent extraction (hexane)**	**Hydrodistillation**	**Supercritical Extraction (CO_2)**
1	rose oxide	0.5	0.0	0.5	0.4
2	isomenthone	5.6	2.1	4.8	3.5
3	linalool	2.7	0.0	4.4	0.1
4	guaia-6,9-diene	5.9	5.5	7.2	8.8
5	citronellyl formate	13.2	6.0	11.1	10.2
6	germacrene-D	2.4	3.2	2.5	4.6
7	geranyl formate	5.5	4.7	3.7	7.9
8	citronellol	26.9	21.3	26.5	24.8
9	geraniol	8.1	10.8	8.4	8.5
10	geranyl tiglate	3.3	3.3	3.1	3.3
11	2-phenylethyl tiglate	1.8	2.2	1.9	1.8
	Total	75.9	59.2	74.2	73.8

was verified that the composition of some components had changed: the amounts of geranyl formate and citronellyl formate were smaller in the control sample (around 2% less), while the content of guaia-6,9-diene and linalool increased 1.2 and 1.7 times, respectively. The remaining composition did not change significantly.

Comparing the hydrodistillation and SFE extraction methods (see Figure 4.14), small differences in the composition were found, except for the content of linalool. The linalool is formed from geraniol when submitted at high temperatures in the presence of water vapour (Guerere *et al.*, 1985). Therefore, the linalool amount in SFE extract was considerably low, while in the solvent extract was completely absent since the extraction was performed at room temperature (around 25 ºC).

SFE extracts were richer in the key components herein considered (see Table 4.3), except for the volatile monoterpenes that are more concentrated in the essential oil. Taking into account that the

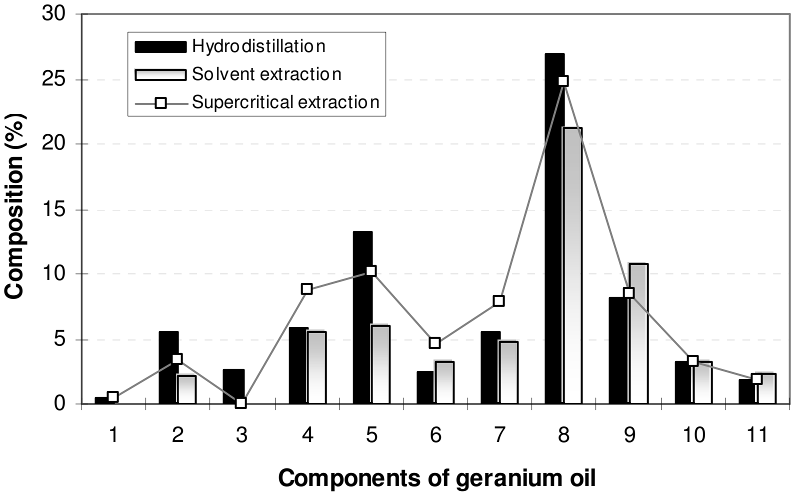


Figure 4.14 Comparison of composition of geranium oil and extracts obtained with different extraction methods. 1. rose oxide; 2. isomenthone; 3. linalool; 4 guaia-6,9-diene; 5. citronellyl formate; 6. germacrene-D; 7. geranyl formate; 8. citronellol; 9. geraniol; 10. geranyl tiglate; 11. 2-phenylethyl tiglate.

contribution of the monoterpenes to the organoleptic quality of the fragrance material is not significant, it can therefore be concluded that supercritical extraction is more efficient for fragrance applications than hydrodistillation.

Considering the solvent extraction technique, the SFE extract was richer in the more volatile components (rose oxide, isomenthone, formates) and hydrocarbon terpenes (guaia-6,9-diene and germacrene D) than the hexane extract. On the other hand, the low volatility components (tiglates) remained approximately the same, while the rose scented components citronellol and geraniol were slightly more concentrated in SFE and SE extract, respectively. Therefore, it can be concluded that SFE was also a more efficient extraction method than solvent extraction.

4.4.2 Effect of operating parameters

In this work, the influence of the operating conditions on the extraction yield, chemical composition and organoleptic quality was considered. The extraction parameters herein studied were: time of extraction, pressure, temperature, plant pre-treatment and operating mode (static/dynamic).

To analyze the influence of parameters on the composition of the SFE extracts, 4 groups of key fragrant components representative of the main types of components were considered:

F1 - the most volatile fraction, constituted by rose oxide and isomenthone

F2 – the characteristic fraction, constituted by guaia-6,9-diene and citronellyl formate (two components characteristic of geranium oil)

F3 – the rose scented fraction, composed of citronellol and geraniol (rhodinol fraction)

F4 – the low volatility fraction, with geranyl tiglate and phenylethyl tiglate.

All the fractions were compared with the essential oil (EO) composition.

Extraction time effect

The SFE yield was little affected by the extraction time, as can be seen in Figure 4.15, being around 0.05% for the reference extraction conditions (shown in Table 4.2).

On the other hand, the composition of extracts had changed, as shown in Table 4.4 and Figure 4.16. The content of the most volatile fraction (F1) showed a tendency to increase with longer extraction time. The amount of the remaining fractions, namely the characteristic fraction (F2), the rose fraction (F3) and the low volatile fraction (F4) displayed a minimum at 30-60 min. It can be concluded that there were two competing phenomena that occurred during extraction time: the reach of equilibrium solubility and the dilution effect of more components being soluble in supercritical CO_2. So, for the more volatile fraction the increase of soluble amount was dominant, while for the others the dilution effect was more important for smaller extraction times, but the increase of soluble amount became dominant for extraction times higher than 60 min.

It is noteworthy that for very long extraction time (180 min) the amount of linalool was higher, as can be seen in in Table 4.4, which indicates that there was a degradation of geraniol into linalool, due to the long heating and exposure to water vapour, as has been already mentioned in section 4.4.1 for explaining the presence of linalool in the hydrodistilled essential oil (Guerere *et al.*, 1985).

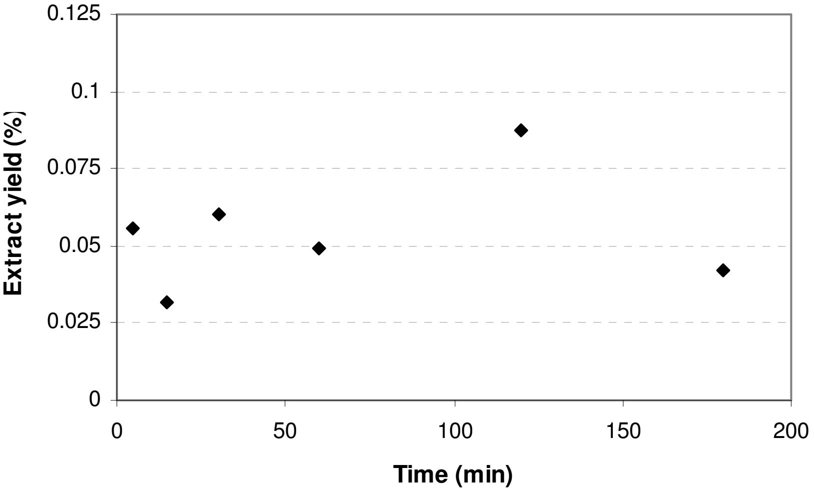


Figure 4.15 Effect of extraction time on SFE yield.

Table 4.4 Percentage composition of SFE extracts obtained at different extraction time and comparison with the composition of geranium essential oil (EO).

		EXTRACTION TIME (min)						
No.	**Component**	**5**	**15**	**30**	**60**	**120**	**180**	**EO**
1	rose oxide	0.25	0.19	0.37	0.31	0.39	0.42	0.46
2	isomenthone	3.67	3.01	3.50	3.36	3.45	4.31	4.81
3	linalool	0.07	0.02	0.06	0.08	0.07	0.17	4.44
4	guaia-6,9-diene	9.12	9.58	8.43	8.49	8.81	7.14	7.23
5	citronelyl formate	9.64	7.82	9.42	8.96	10.20	9.70	11.08
6	germacrene D	4.47	4.99	4.14	4.26	4.60	3.71	2.49
7	geranyl formate	8.56	6.49	5.92	6.57	7.87	9.37	3.70
8	citronellol	22.82	23.89	20.37	20.60	24.78	27.08	26.52
9	geraniol	9.10	9.11	7.92	7.59	8.52	10.84	8.44
10	geranyl tiglate	3.55	4.61	3.06	3.10	3.26	2.92	3.08
11	phenylethyl tiglate	1.99	2.24	1.63	1.56	1.80	2.16	1.94
	Total	73.24	71.95	64.81	64.88	73.75	77.82	74.18

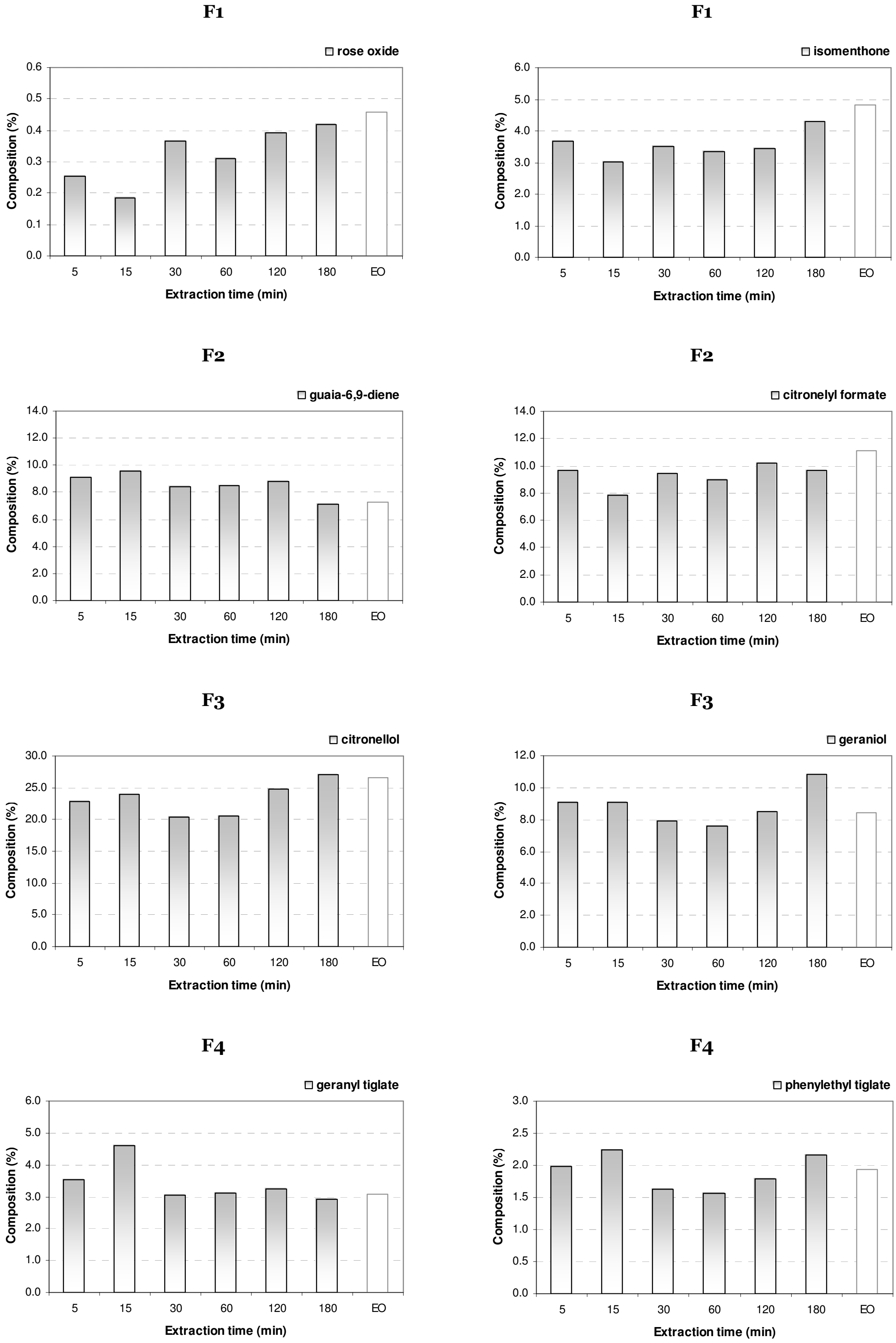


Figure 4.16 Variation of composition of SFE extracts with extraction time and comparison with geranium oil (EO), considering different fractions: F1-the most volatile fraction; F2–the characteristic fraction; F3-rose odour fraction and F4-low volatile fraction.

Pressure effect

The SFE yield was significantly influenced by the extraction pressure, as can be seen in Figure 4.17. In fact, increasing the extraction pressure from 80 to 160 bar, the yield increased more than 10 times. The higher CO_2 density at high pressures increases its solvent power and therefore more substances were extracted.

The study of pressure effect on composition is shown in Table 4.5 and in Figure 4.18. The most volatile fraction (F1) increased with pressure, as can be seen in Figure 4.18-F1. However, the rose fraction (F2) and the low volatility fraction (F4) amount decreased, more significantly for F4. Considering the characteristic fraction, citronellyl formate followed the increasing trend shown by fraction F1 (most volatile), while guaia-6,9-diene did not change significantly. These results show that only for the more volatile components, the increase of soluble amount with increasing pressure was significant.

Temperature effect

As temperature increased, the extraction yield decreased (Figure 4.19). It was observed that increasing extraction temperature from 40 ºC to 100 ºC the yield decreased 3 times. This fact reveals that the effect of decreasing the fluid density and thus its solving power is dominant over the increase of vapour pressure of components.

On the other hand, comparing the compositions shown in Table 4.6 and graphically in Figure 4.20, it can be seen that the temperature affected differently the components behaviour. Rose oxide in F1 and citronellyl formate in F2 had a maximum amount in the extract of 80 ºC and citronellol in F3 had a maximum at 60 ºC. By contrast, geranyl and phenylethyl tiglates inF4 fraction showed a minimum at 80 ºC, as well as geraniol in F3, although it is little pronounced. In the case of isomenthone in F1, the effect of temperature is not evident. It can be concluded that for those components that showed a maximum in composition, the effect of increasing vapour pressure with increasing temperature was dominant over the loss of solving power of supercritical CO2, happening the opposite for the ones that showed a minimum.

Looking at linalool composition in Table 4.6, its amount in spite of still low compared with the essential oil amount, was increasing with the increase of extraction temperature, especially at the

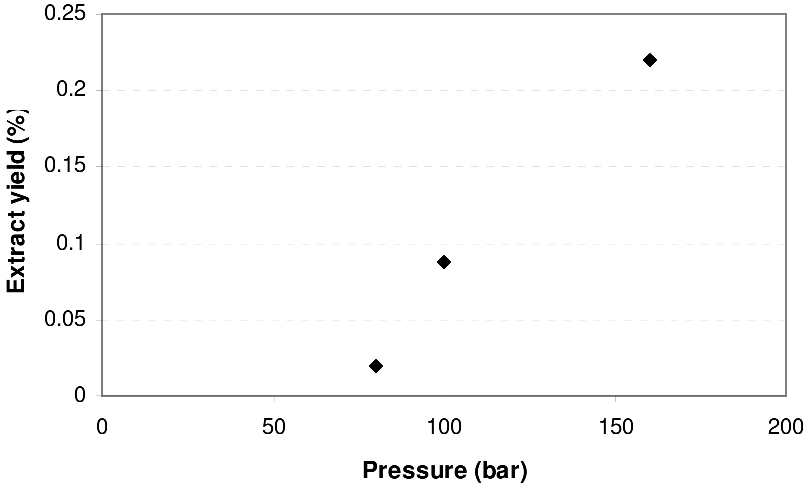


Figure 4.17 Effect of pressure on SFE yield.

Table 4.5 Percentage composition of SFE extracts obtained at different extraction pressures and comparison with the composition of geranium essential oil (EO).

		PRESSURE (bar)			
No.	**Component**	**80**	**100**	**160**	**EO**
1	rose oxide	0.00	0.39	0.50	0.46
2	isomenthone	1.13	3.45	3.70	4.81
3	linalool	0.00	0.07	0.07	4.44
4	guaia-6,9-diene	9.53	8.81	9.94	7.23
5	citronelyl formate	6.50	10.20	11.54	11.08
6	germacrene D	4.94	4.60	5.62	2.49
7	geranyl formate	6.45	7.87	7.32	3.70
8	citronellol	26.57	24.78	21.73	26.52
9	geraniol	12.28	8.52	6.82	8.44
10	geranyl tiglate	5.88	3.26	3.66	3.08
11	phenylethyl tiglate	2.93	1.80	1.70	1.94
	Total	76.22	73.75	72.59	74.18

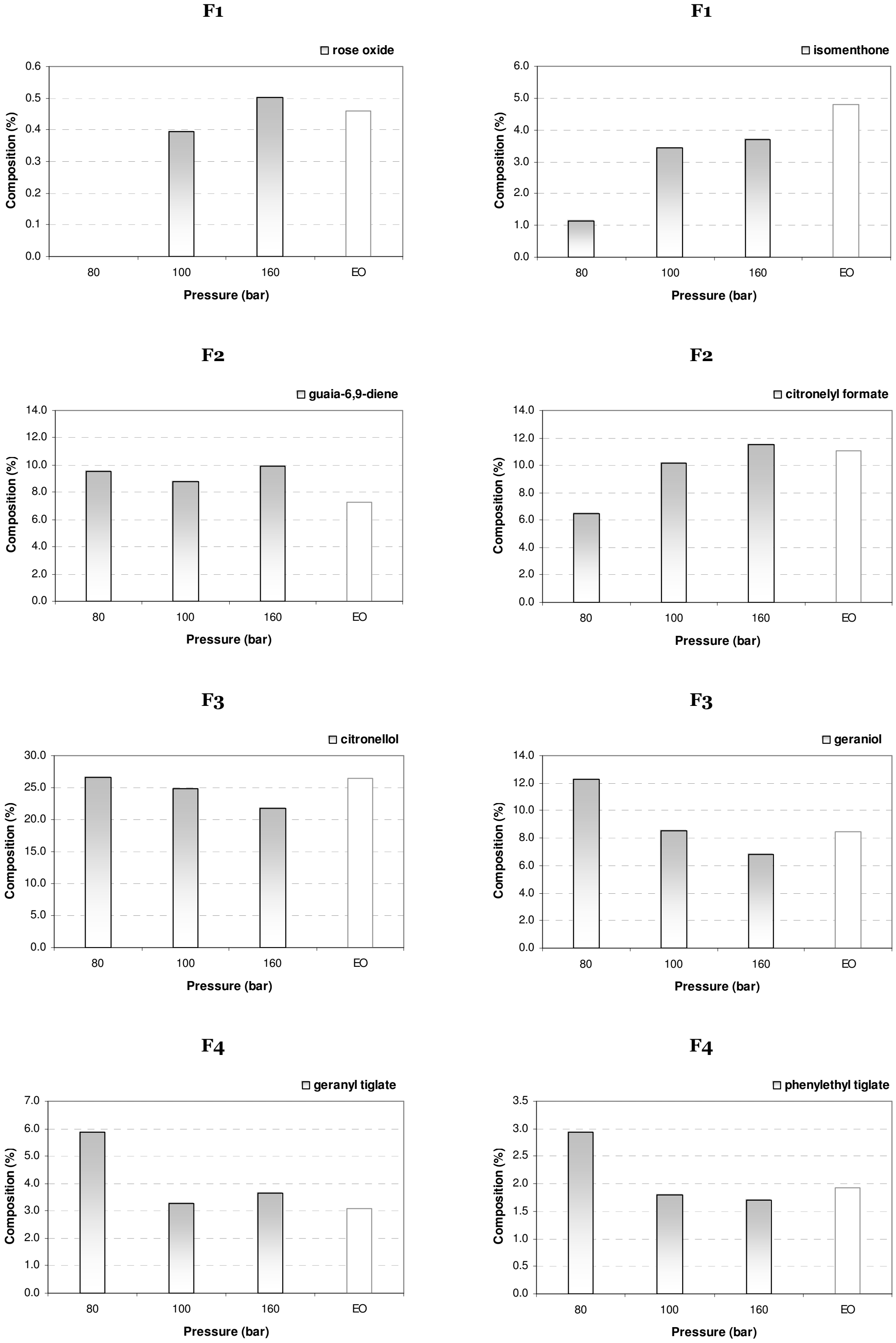


Figure 4.18 Variation of composition of SFE extracts with extraction pressure and comparison with geranium oil (EO), considering different fractions: F1-the most volatile fraction; F2–the characteristic fraction; F3-rose odour fraction and F4-low volatile fraction.

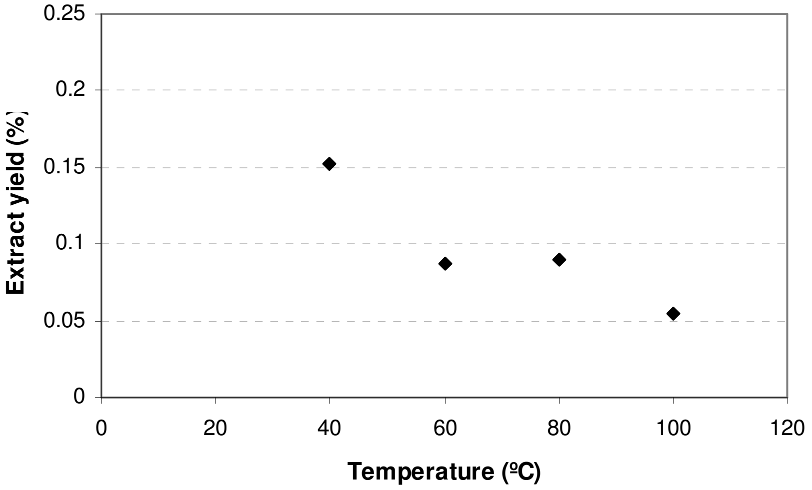


Figure 4.19 Effect of temperature on SFE yield..

Table 4.6 Percentage composition of SFE extracts obtained at different extraction temperatures and comparison with the composition of geranium essential oil (EO).

		TEMPERATURE (°C)				
No.	**Component**	**40**	**60**	**80**	**100**	EO
1	rose oxide	0.30	0.39	0.45	0.28	0.46
2	isomenthone	4.12	3.45	4.89	3.01	4.81
3	linalool	0.06	0.07	0.26	0.69	4.44
4	guaia-6,9-diene	8.88	8.81	9.78	11.81	7.23
5	citronelyl formate	10.21	10.20	13.08	9.08	11.08
6	germacrene D	4.61	4.60	4.35	4.87	2.49
7	geranyl formate	7.44	7.87	11.00	8.42	3.70
8	citronellol	23.64	24.78	20.90	18.77	26.52
9	geraniol	8.75	8.52	7.82	8.14	8.44
10	geranyl tiglate	3.24	3.26	2.53	4.03	3.08
11	phenylethyl tiglate	1.80	1.80	1.27	1.84	1.94
	Total	73.04	73.75	76.32	70.95	74.18

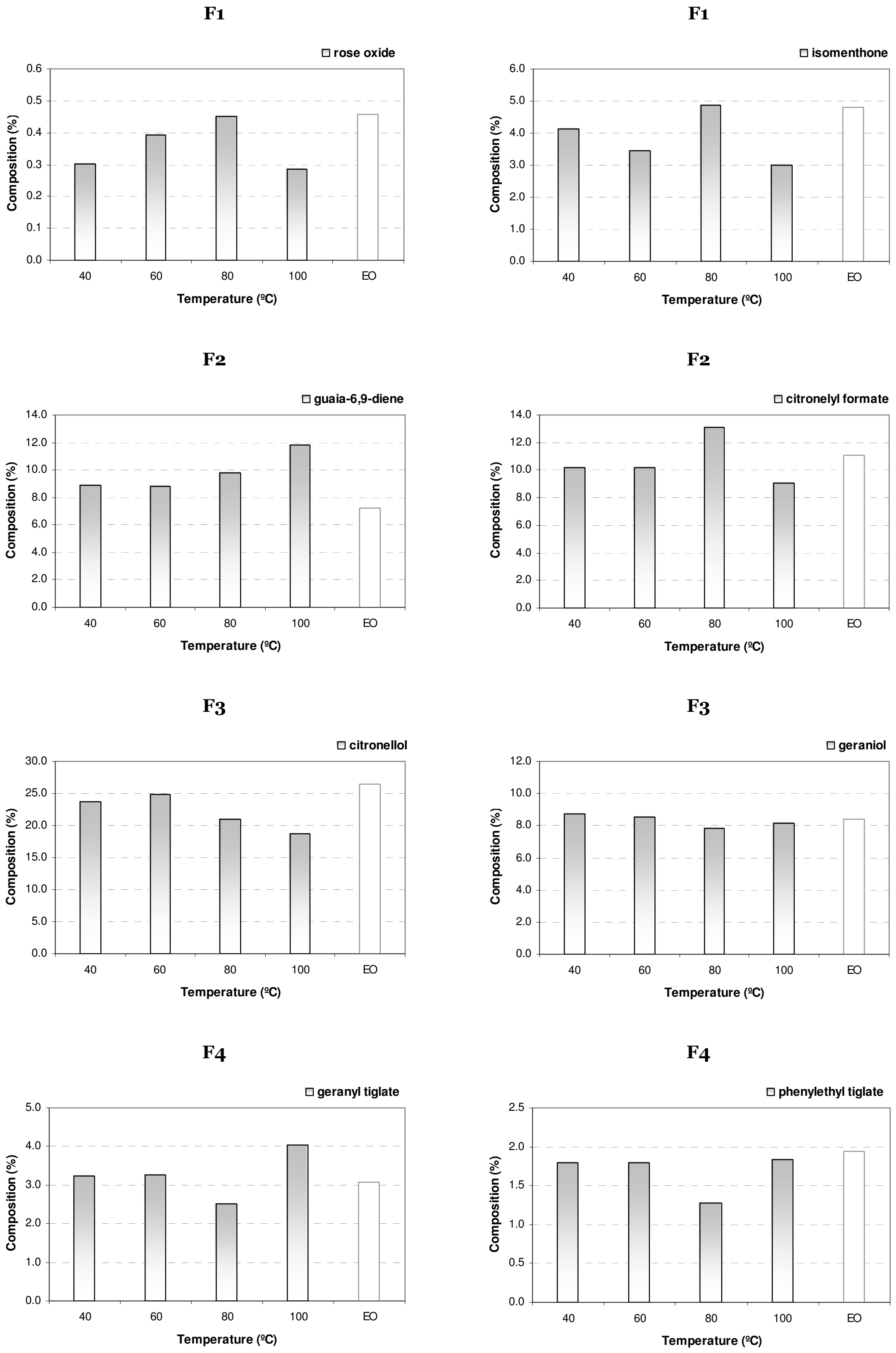


Figure 4.20 Variation of composition of SFE extracts with extraction temperature and comparison with geranium oil (EO), considering different fractions: F1-the most volatile fraction; F2–the characteristic fraction; F3-rose odour fraction and F4-low volatile fraction.

higher temperatures of 80 and 100 °C, due to the effects already mentioned and described by Guerere *et al.* (1985).

Pre-treatment of plant effect

The extraction yield obtained by using ground frozen plant was 0.11%, which is 25% higher than the yield obtained using cut fresh plant (0.088%). This is explained by the fact that grounding the plant, the contact surface area is increased, improving the extraction. Note that the increase of yield means that more material is extracted, but not necessarily that there is an increase in the amount of volatile fraction yield because non-volatiles are also extracted.

The influence of this parameter on composition is shown in Table 4.7 and Figure 4.21. The content in

Table 4.7 Percentage composition of SFE extracts obtained with different plant pre-treatments and comparison with the composition of geranium essential oil (EO).

		PLANT PRE-TREATMENT		
No.	**Component**	**Cut fresh**	**Ground frozen**	**EO**
1	rose oxide	0.39	0.29	0.46
2	isomenthone	3.45	3.24	4.81
3	linalool	0.07	0.10	4.44
4	guaia-6,9-diene	8.81	11.44	7.23
5	citronelyl formate	10.20	10.40	11.08
6	germacrene D	4.60	5.93	2.49
7	geranyl formate	7.87	10.11	3.70
8	citronellol	24.78	19.04	26.52
9	geraniol	8.52	7.49	8.44
10	geranyl tiglate	3.26	3.70	3.08
11	phenylethyl tiglate	1.80	1.71	1.94
	Total	73.75	73.43	74.18

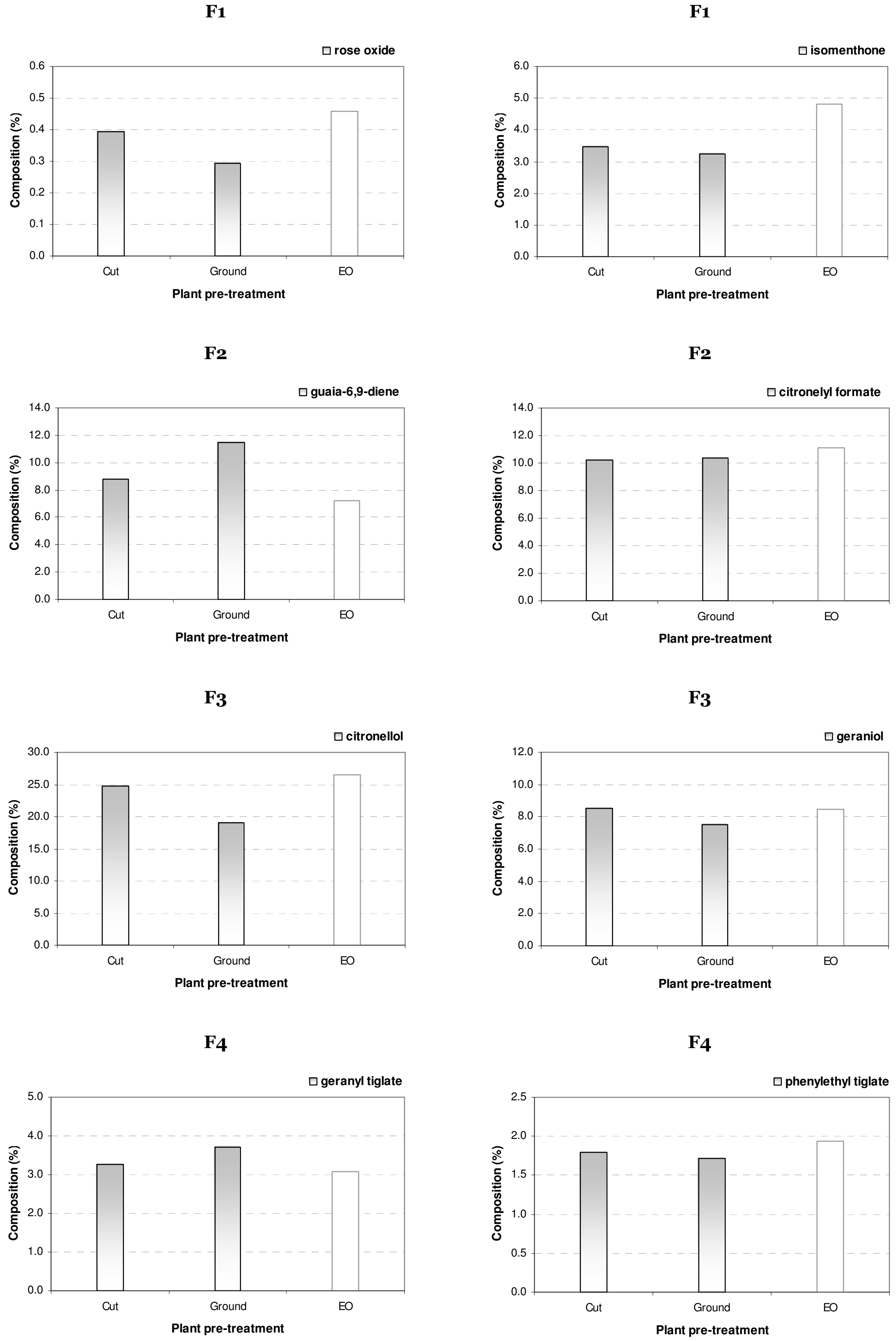


Figure 4.21 Variation of composition of SFE extracts with plant pre-treatment (cut fresh and ground frozen) and comparison with geranium oil (EO), considering different fractions: F1-the most volatile fraction; F2–the characteristic fraction; F3-rose odour fraction and F4-low volatile fraction.

the most volatile components (F1) and rose scented fraction (F2) decreased 8% and 20%, respectively while the amount of guaia-6,9-diene in F3 increased 27%, as can be seen in Figure 4.21. For the remaining components in fractions F4 and citronellyl formate, the effect was not significant. This result revealed that there was loss in the volatile components during the process of grinding, even though the plant was ground frozen, while the non-polar components that have more affinity to wax material in the plant, are better extracted when the contact area with the solvent is bigger.

Operating mode effect

The extraction yield using dynamic operating mode was 0.13%, while the yield obtained in static SFE extraction was 0.088%. The yield increased 43% using the dynamic mode.

The composition variation is shown in Table 4.8 and Figure 4.22. The dynamic mode favored the extraction of the more volatile fraction (F1) and the characteristic fraction (F2). For the rose scented fraction (F3) and low volatile fraction (F4), their amount decreased using the dynamic mode.

Table 4.8 Percentage composition of SFE extracts obtained with different operating modes and comparison with the composition of geranium essential oil (EO).

		OPERATING MODE (time = 15 min)		
No.	**Component**	**Dynamic**	**Static**	**EO**
1	rose oxide	0.25	0.19	0.46
2	isomenthone	4.44	3.01	4.81
3	linalool	0.09	0.02	4.44
4	guaia-6,9-diene	10.48	9.58	7.23
5	citronelyl formate	10.07	7.82	11.08
6	germacrene D	5.23	4.99	2.49
7	geranyl formate	8.24	6.49	3.70
8	citronellol	20.79	23.89	26.52
9	geraniol	8.12	9.11	8.44
10	geranyl tiglate	3.33	4.61	3.08
11	phenylethyl tiglate	1.78	2.24	1.94
	Total	72.82	71.95	74.18

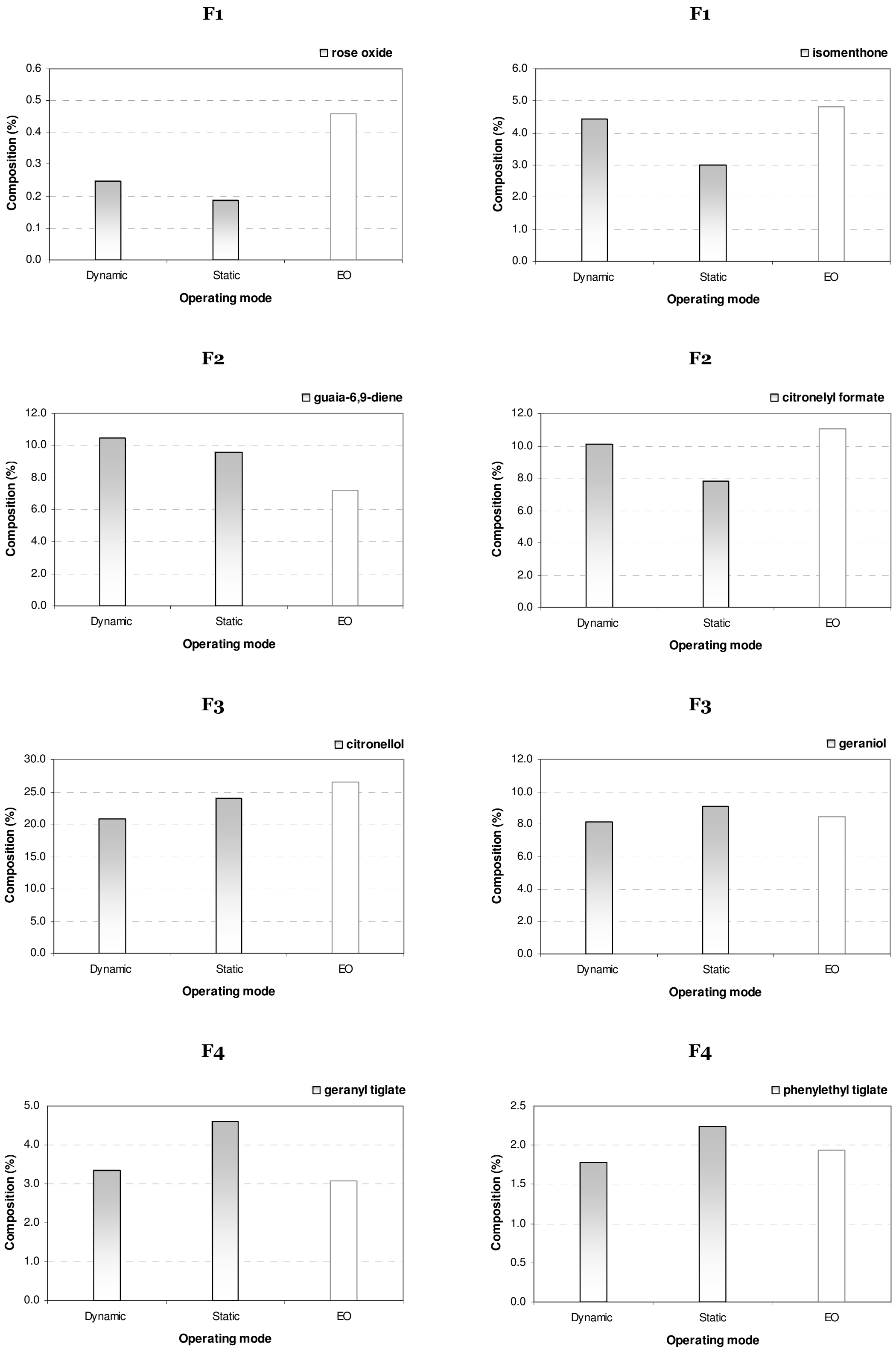


Figure 4.22 Variation of composition of SFE extracts with operating mode (static/dynamic) and comparison with geranium oil (EO), considering different fractions: F1-the most volatile fraction; F2–the characteristic fraction; F3-rose odour fraction and F4-low volatile fraction.

4.4.3 Organoleptic description

The absolutes from SFE extracts showed a pale yellow - yellow colour, having a floral-fruity natural clean fresh character, resembling the actual plant scent but with the floral-fruity note more striking. The 'still' or 'tea' off notes (boiled plant), characteristic of geranium essential oil but not desired, were not noticed in SFE absolutes. The floral character is due to the components geraniol and citronellol, while the fruity nuances derive from their formates, acetates and tiglates and the freshness is imparted by isomenthone.

The colour was more intense for the extracts obtained at lower temperature (40 °C), higher pressure (160 bar), using smaller particle size (ground plant) and dynamic operating mode.

The absolute from SFE extract obtained at very high pressure presented a greener odour nuance due to its higher amount of rose oxide (strong odour reminiscent of geranium and carrot leaves (Bauer *et al.*, 1997)).

The SFE extraction at 100 °C resulted in an absolute with an odour closer to the essential oil obtained by hydrodistillation at the same temperature (boiling temperature of water). On the other hand, the absolute corresponding to SFE extract obtained at 80 °C had a fresher smell, with a minty nuance more intense due to the higher contents in isomenthone.

The smelling strips were practically not stained, so the non-volatiles content was low.

The concrete was a yellow paste, with a sweet floral balsamic odour, most probably due to the presence of waxes.

Comparing SFE absolutes with other natural materials derived from geranium, namely essential oil and absolute from hexane extract, the SFE absolute showed a higher organoleptic quality, either in terms of odour - much more natural and fresh-floral-fruity, without 'tea' cooked off notes-, as well as in terms of colour, where its pale yellow-yellow colour is a common colour found in fragrance ingredients and is perfectly acceptable for most perfumery applications (the essential oil is green coloured and the absolute from hexane extract has green-brownish colour).

4.5 Conclusions

A small-scale homemade equipment for supercritical extraction was designed and constructed in this work. The clean technology of CO2 supercritical extraction was employed for the extraction of rose geranium plant (*Pelargonium sp*) to produce a high quality natural material that can be used in perfumery.

The design of SCRITICAL has resulted in a compact, versatile and portable equipment, which requires less laboratory space to accommodate. As it is compact, there is minimum tubing and therefore minimum dead volumes. The control panel makes easy the control and monitoring of the supercritical extraction process. The heating using an oven is more economical since it does not require a jacketed extractor and thermal baths.

The main advantage of this equipment is the fact that it enables higher flow rates and extraction volumes compared with to the available commercial laboratory equipments. Moreover, it is highly versatile since it is possible to use different extractor and separator volumes and add extra regulation valves, extraction and separation vessels, for example.

The limitation of this equipment is that the piston pump does not allow long continuous operation times due to the friction and heating that can damage the piston mechanism. Considering this, the study of the influence of operating parameters on extraction performance was carried out in most cases in the static (equilibrium) mode. A diaphragm or membrane pump would be more appropriate but it is considerably more expensive for the same range of flowrates. Eventually more depressurization steps should be introduced by the use of more regulation valves in order to have lower pressure drops and so prevent more efficiently freezing due to Joule-Thomson effect.

The stabilization time in the start of SFE operation (for equilibration of temperature and pressure) can also be considerably reduced if the system is previously pressurized, before feeding compressed CO2.

The influence of operating parameters on yield, chemical composition and organoleptic quality of CO_2 extracts was studied, namely time of equilibrium, pressure, temperature, plant pre-treatment and operating mode. It was shown that extraction time did not have a significant influence, the yield increased with increase of the pressure and decrease of temperature. The yield was higher using ground frozen plant and the dynamic mode. On the other hand, the composition of extracts must be considered since it will determine the odour and other organoleptic qualities of the extracts. A compromise must be found between maximizing the yield and maximizing the concentration of fragrant key components. The optimum SFE conditions were then found to be: extraction time of 15-30 min, temperature of 40 °C, pressure of 90-100 bar, cut fresh plant and dynamic mode.

The chemical analysis revealed that although roughly the same components were extracted, from the organoleptic point of view, the supercritical absolutes showed a considerable high quality in terms of odour and colour, with very fresh natural floral-fruity character and a pale yellow colour, most suitable for the use in perfumery.

The design and construction of SFE set-up has enabled to develop expertise in supercritical CO_2 extraction, with the possibility in the future to apply this clean technology to other natural materials. On the other hand, using rose geranium as a case study, it was possible to apply a high-level technology to the production of high-added value materials with potential for industrial scale-up in Portugal.

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CHAPTER 5

REFINING OF NATURAL MATERIALS USING PREPARATIVE LIQUID CHROMATOGRAPHY

There is an increasing interest in natural products in perfume industry due to its high olfactory quality and to impart some “naturalness” and complexity to the perfume compositions.

This chapter has the objective to use preparative liquid chromatography to fractionate essential oils, in order to produce a higher value material for F&F applications. This method will be applied to the deterpenation of citrus oils. Citrus terpeneless oils are employed as top notes in perfumes, having better stability and solubility in ethanol than citrus oils with terpenes. Moreover, the citrus fruits, namely lemon, are grown easily in Mediterranean region, including Portugal, constituting this way a natural resource with commercial potential to explore. A model of preparative chromatography was also developed to optimize the separation conditions.

5.1 Introduction

Citrus oils are constituted up to 95% by monoterpenes, mainly limonene. The worldwide production of citrus is around 20,000 tons per year, of which 2500 tons are of lemon oil (Pybus and Sell, 1999). They are used as a flavour in food industry and as a fresh top note ingredient in perfumery (Bauer *et al.*, 1997).

However the important aroma-determining components of citrus oils are oxygenate components that are present in minor quantities (3 to 5%) (Bauer *et al.*, 1997), such as citral (strong lemon odour), decanal and linalool, whilst limonene and other hydrocarbon monoterpenes act as carriers. Moreover, as the citrus oils are obtained by expression, there is also a small fraction of non-volatiles of waxes, pigments and other high-molecular mass components.

In practice, the monoterpene hydrocarbon fraction is partially removed – deterpenation - to improve the organoleptic quality of the oils, namely to prevent rancid odours and skin irritation, as well as to improve its solubility in polar solvents like alcohol. The non-volatile compounds should also be removed, by decantation at low temperature for example, since they give rise to turbidity and some of them have photosensitising activity, such as bergaptene in bergamot oil (Bauer *et al.*, 1997).

The terpeneless oils are commercialized as folded oils or x-fold Citrus oil, being x the concentration factor (usually 5, 10 or 20). These oils should have at least half of the quantity of the original hydrocarbon monoterpenes, most of it being limonene (Imison and Unthank, 2000).

Terpeneless oils are therefore high-added value raw materials, more soluble in ethanol, more stable to oxidation, light and temperature and present stronger and “rounder” odour than ordinary oils.

On the other hand, the extracted terpenes are used to produce natural identical oils or synthetics, such as Lyral® and Iso E Super® obtained from β-pinene, linalool and α-terpineol from α-pinene, L-carvone from D-limonene and musk Tonalid® from p-cymene (Pybus and Sell, 1999). Moreover, the terpene fraction is used to impart naturalness to perfume compositions of aroma chemicals and as industrial solvents.

There are several industrial processes to perform deterpenation of essential oils (Bauer *et al.*, 1997; Curtis and Williams, 1994):

- fractional distillation, yielding lower boiling fractions rich in monoterpenes and higher boiling fractions rich in oxygenated compounds;
- counter-current liquid-liquid extraction using one polar solvent and one non-polar solvent, coupled with vacuum distillation to recover the solvents;
- solvent extraction with a polar solvent, i.e. triacetin, followed by vacuum distillation to remove the solvent;
- supercritical fluid extraction and/or fractionation;
- adsorption methods, namely on silica packing beds.

The liquid chromatography presents some advantages over the other methods, such as: simplicity of the equipment, minimal exposure to air, easy operation and usually at ambient temperature (avoiding thermal degradation), enables the separation of non-volatile materials (which is not possible by GC), provides high sample recovery rates and high purities (Hostettmann *et al.*, 1997; Pino, 1992), minimizing the undesired removal of oxygenated components with the terpene fraction as happens in distillation (Kirchner and Miller, 1952). Since the essential oils are very complex mixtures, the liquid chromatography is used either to separate them into fractions of compounds like oxygenated, monoterpenes, sesquiterpenes, or to separate one of these fractions into its constituents. Moreover, chromatography separation is preferable rather than solvent extraction because it is more selective to aroma components. On the other hand, non-volatile components deactivate the adsorbent by fouling the pores and active sites, so they must be previously removed. Unless the aim is to remove undesirable constituents by irreversible binding to adsorbent material, as happens at industrial-scale adsorption processes applied to juices, sugar and wines (Gehrke *et al.*, 2000).

The silica unmodified phase is the adsorbent most used for applications in natural products due to its lower cost and wide range of solvents as eluents, easy removal of solvents after fractionation and higher flow rates (Hostettmann *et al.*, 1997). This stationary phase adsorbs strongly the polar compounds, while the monoterpenes to remove are eluted through out the chromatographic column (Imison and Unthank, 2000). Afterwards the retained polar compounds are desorbed and recovered. Sometimes to accomplish desorption, it is necessary to change the solvent to ethanol for example.

The deterpenation process is more advantageous by adsorbing the polar components rather than the non-polar components, since the polar ones exist in smaller amount and so require considerably less quantity of adsorbent material to accomplish the separation for a given feed loading (Imison and Unthank, 2000).

5.1.1 Deterpenation using adsorption

In 1952, Kirchner and Miller (Kirchner and Miller, 1952) proposed the use of chromatographic method to commercial preparation of terpeneless essential oils, after they noticed when working with citrus flavours that oxygenated constituents where tightly adsorbed on the bottom of silicic acid chromatostrips, while hydrocarbons were readily carried away with hexane. They studied this application to eight essential oils: orange, grapefruit, lemon, clove, peppermint, petitgrain, bay and cedarwood. The terpeneless oils obtained by adsorption showed an improved delicate odour comparing to the original oils, free of hydrocarbons.

Pino (Pino, 1992) carried out an interesting investigation on deterpenation of black pepper oil by chromatography on silica gel. The author concluded that the proportion 1:1 adsorbent/essential oil ratio represented a good compromise between separation and cost. Moreover, a sensorial analysis was performed, showing that terpeneless oils have a similar odour profile to original, except for an intensification of woody note (oxygenated sesquiterpenes) and small decrease of resinous, pungent and piney notes.

Dugo et al. (Dugo *et al.*, 1998) used normal-phase chromatography on silica adsorbents to isolate and quantify the non-volatile oxygen heterocyclic compounds of lemon oil, at analytical and semi-preparative scale.

Gehrke et al. (Gehrke *et al.*, 2000) studied the recovery of volatile flavour compounds from aqueous media by selective in situ adsorption of polar terpenoids in the presence of less polar terpenes, in order to increase yields in bioprocesses for flavours and fragrances area. They designed and characterized derivatized, macroporous cross-linked polystyrene divinylbenzene resins (derivatives of Amberlite XAD16), using two binary model systems: (-)-limonene/(-)-carvone and (-)-α-pinene/(-)-borneol. They found one adsorbent more selective to polar component than to monoterpene, in organic phase (n-hexane), keeping its capacity and selectivity after repeated experiments.

Imison and Unthank (Imison and Unthank, 2000) studied the fractionation of orange oil by using supercritical CO2 extraction. They screened several adsorbents, finding that silica gel had the highest adsorptive capacity. However, with reuse the adsorbent was loosing capacity and selectivity because of fouling of adsorbent pores with waxes and other high molecular weight components. This project showed that the use of SFE technology to orange oil was not economically viable, since the product does not have enough value to compensate the high price of this technology. Nevertheless, there are several publications on the SFE deterpenation of citrus (Benvenuti *et al.*, 2001; Budich *et al.*, 1999; Goto *et al.*, 1997; Reverchon and Iacuzio, 1997; Sato *et al.*, 1998a; Sato *et al.*, 1998b).

Ogawa et al. (Ogawa *et al.*, 2002) described two chromatographic techniques for the analysis of volatile hydrocarbon and oxygenated monoterpenes in orange oil: capillary liquid chromatography (CLC) and micellar electrokinetic chromatography (MEKC). The adsorbent used in the CLC was reversed-phase fused-silica RP-18. The components analysed were mircene, α- and β-pinene, α-terpineol, linalool, citral and carvone. As the reversed-phase is selective to non-polar components, the polar fractions eluted first with poor resolution, whilst the non-polar components were more retained and had better resolution. Wineski et al. (Wisneski *et al.*, 1984) also developed a method based on HPLC on reversed-phase C18 to determine trans-cinnamaldehyde in perfumes.

The fractionation of essential oils by liquid chromatography considered in these published works were carried out only at analytical scale. In this work, the deterpenation of essential oils by preparative liquid chromatography to produce terpeneless essential oils will be studied.

5.2 Preparative chromatography

Preparative chromatography is a purification process that aims to separate mixtures into pure components or simpler mixtures (fractions) in order to obtain a product, whilst analytical chromatography relies on separation to enable identification of components and so the recovery of the sample (or fractions) is not important. The quantity of sample to be separated is also different, with amounts of product below 0.5 mg for analytical scale and for preparative chromatography can go up to several grams or kilograms at industrial-scale (Hostettmann *et al.*, 1997).

Naturally, the column is the most important part in chromatographic separation processes. The main parameters used for design and characterization of performance of chromatographic column are presented schematically in Figure 5.1. These parameters must be optimized in order to get the best separation with the best possible efficiency (Colin, 1993; Cox, 1995). The selection of the of the mobile phase is one of the most critical steps concerning the optimisation of the process, which should compromise between selectivity, loadability, separation time/viscosity, ease of regeneration, ease of removal from product and the solvent cost. However, the choice of the solvent composition must be done by experimentation because it is not possible yet to predict the influence of solvent composition on selectivity (Nicoud and Colin, 1990).

The separation process performance is evaluated by four performance criteria: productivity or production rate, solvent consumption, purity and recovery. The purity is wanted as high as possible, but must compromise with other variables, and so usually constitutes one product requirement that is established by the market needs or process limitations. The recovery is related to the yield of the process and is more relevant when dealing with expensive raw materials, as in the case of pharmaceuticals. The operating costs are mainly determined by solvent consumption and productivity. The lower the solvent consumption, the lower are the costs of consumables (solvent amount) and costs associated with removal of solvent to concentrate de product and its disposal as hazardous chemicals

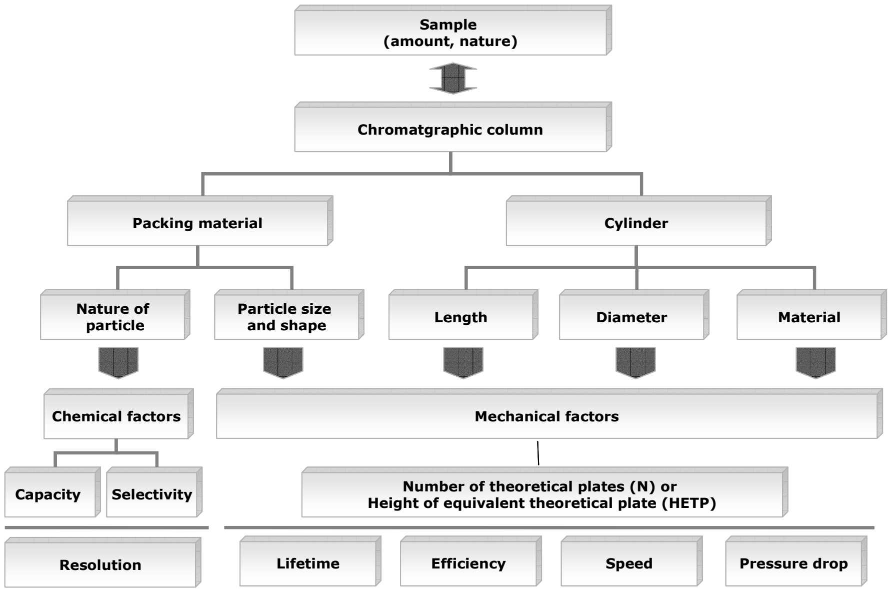


Figure 5.1 The parameters involved in design of chromatographic columns and their contribution to separation performance (adapted from Hostettmann et al.,1997).

in the end. The higher the productivity, the shorter is the time of process and therefore the utility and labour costs are decreased. The optimisation of a chromatographic separation is based on these criteria. This optimisation must be done case by case because there is no general procedure for a particular separation problem (Kaminski and Reusch, 1988).

Recycling liquid chromatography is one of the most studied operating modes in chromatographic separation (Bailly and Tondeur, 1982; Charton *et al.*, 1994; Crary *et al.*, 1989; Grill and Miller, 1998). Recycling enhances the recovery and purity of single-pass separations that are not completely resolved, such as separating two or more components with close retention times. The column effluent is sent back at the inlet of the column, so that the material poorly separated is recirculated until the components have the required purity.

The practical effect of recycling is to simulate the increasing the column length, without spending more packing material and using bigger columns. The mixing effect that results of the passage of solute through the pump, leading to peak band broadening, can be neglected if the internal pump volume is considerably smaller than the column volume.

The recycle may be total if done in closed loop, or partial if only the fraction that has the mixed components is recycled to be further separated (Coq *et al.*, 1981; Heuer *et al.*, 1999). Figure 5.2 illustrates partial or shave recycling. This recycling can be combined with a new injection (Grill, 1998). The pure solvent is also recycled so that the solvent consumption is smaller.

Increased productivity is obtained by overloading the column (Neue, 1997). Mass or concentration overloading is also advantageous for the separation of minor components in a mixture, since it is obtained a rich fraction of the minor compound that is then recycled to obtain the pure products (Hostettmann *et al.*, 1997).

In this work, a model of the chromatographic separation with recycling is developed in order to optimise the fractionation of essential oils. The model will be used to select the best mobile phase to promote the aimed separation.

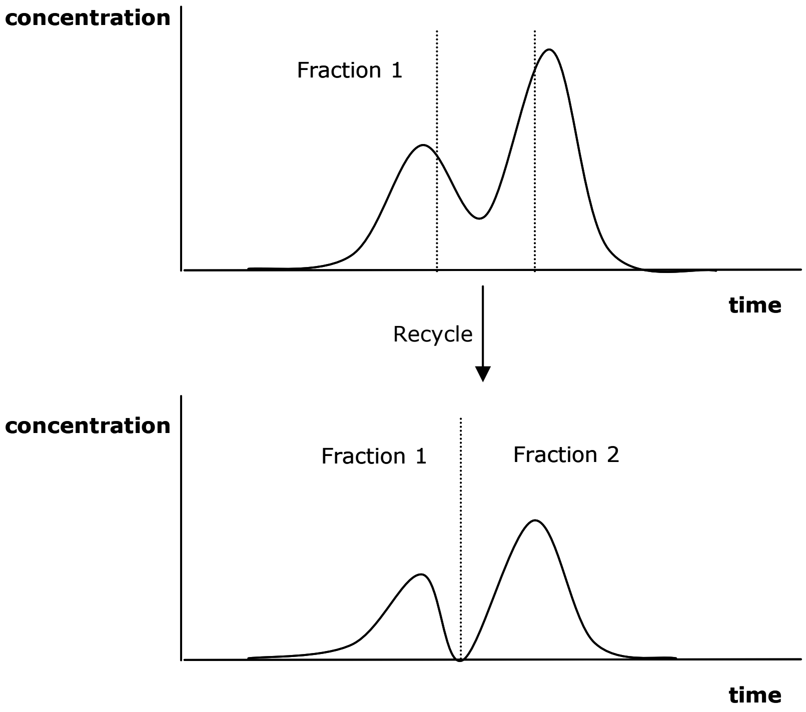


Figure 5.2 Chromatographic separation with partial recycling.

5.3 Description of the preparative chromatography model

5.3.1 Model equations

The simulation of chromatographic separation is based on the equilibrium - dispersion model.

The mass balance for species i is given by (Guiochon *et al.*, 1988):

$$\varepsilon\, D_{ax} \frac{\partial^2 C_i}{\partial z^2} - \varepsilon\, u\, \frac{\partial C_i}{\partial z} = \varepsilon\, \frac{\partial C_i}{\partial t} + (1-\varepsilon)\frac{\partial q_i}{\partial t} \qquad (5.1)$$

where the subscript i refers to the species in the mixture, C_i is the bulk concentration of species i in the mobile phase (g/l), q_i is the adsorbed phase concentrations of species i (g/$l_{adsorbent}$) in equilibrium with the bulk liquid concentration C_i, z is the axial coordinate (m), t is the time variable (s), ε is the bed porosity, u is the interstitial fluid velocity (m/s), D_{ax} is the axial dispersion coefficient (m^2/s).

The equilibrium isotherm relates the concentration adsorbed in the stationary phase material and the concentration in the mobile phases. If the isotherm is linear, the relation is:

$$q_i = K_i \; C_i \tag{5.2}$$

where K_i is the linear isotherm parameter.

Replacing equation (5.2) in (5.1) and rearranging:

$$D_{ax} \frac{\partial^2 C_i}{\partial z^2} - u \; \frac{\partial C_i}{\partial z} = \left[1 + \frac{1-\varepsilon}{\varepsilon} K_i \right] \frac{\partial C_i}{\partial t} \tag{5.3}$$

Introducing the dimensionless variable for space $x = {}^{z}\!/_{L}$, it is obtained:

$$\frac{1}{Pe} \frac{\partial^2 C_i}{\partial x^2} - \frac{\partial C_i}{\partial x} = \tau \; \left[1 + \frac{1-\varepsilon}{\varepsilon} K_i \right] \frac{\partial C_i}{\partial t} \tag{5.4}$$

where the Peclet number, Pe is given by $Pe = {}^{u \; L}\!/_{D_{ax}}$ and the space time is given by $\tau = {}^{L}\!/_{u}$.

The Peclet number (Pe) can be estimated by the following relation (Chung and Wen, 1968):

$$Pe = Pe_P \left(\frac{L}{d_P} \right) \tag{5.5}$$

with

$$Pe_P = 0.20 + 0.11 Re_P \tag{5.6}$$

and

$$Re_P = \frac{\rho \; u \; d_P}{\eta} \tag{5.7}$$

where Re_p is the Reynolds number, L is the column length (m), ρ is the solvent density (kg/m^3), η is the solvent viscosity (Pa·s), u is the fluid velocity (m/s) and d_p (m) is the particle diameter.

Considering that initially the column contains no solute, the initial condition is:

$$C_i(t=0,x)=0 \tag{5.8}$$

The boundary conditions depend on the mode of operation are given by:

Column inlet:

Injection mode: $$C_i(t,x=0)=C_{0i}, \quad 0<t<t_{inj} \tag{5.9}$$

Elution mode: $$C_i(t,x=0)=0, \quad t>t_{inj} \tag{5.10}$$

Recycle mode: $$C_i(t,x=0)=C_i(t,x=1), \quad t_1<t<t_2 \tag{5.11}$$

Column outlet:

$$\frac{\partial C}{\partial x}(t,x=1)=0 \tag{5.12}$$

with C_{0_i} as the initial concentration of component i in the sample injected, t_{inj} as the injection time and t_1, t_2 corresponding to the recycling time interval. In the recycling mode, it is assumed that the transference of the fraction to recycle from the column outlet to the column inlet is instantaneous. The boundary condition at the column outlet corresponds to the assumption of a semi-infinite bed.

5.3.2 Definition of operation modes

The different operation modes are established based on the specification of switching times. These switching times are determined using two variables: the outlet concentration of each component i, C_{Li}, and its instantaneous purity (PUR_i) defined as:

$$PUR_i = \frac{C_{L\,i}}{\sum_{j=1}^{N} C_{L_j}} \times 100 \qquad (5.13)$$

After injection of the sample, there is an elution period until the first fraction comes out of the column. The non-polar fraction is the less retained on silica and is the first to elute.

The first switching time is established when the outlet concentration is above a minimum set value ($C_{\min}$). The collection of the first fraction starts if simultaneously the outlet concentration is above $C_{\min}$ and if the instant purity is higher than the aimed value ($PUR_{\min}$). Otherwise, if purity does not comply the purity condition but $C > C_{\min}$, then the fraction is recycled. The concentration and purity at the outlet of the column are thus continuously monitored in order to determine the operating mode.

Mathematically the operation modes are defined as follows:

Mode 1 – elution: none of the solutes is being obtained

$$C_{L\,i} < C_{\min} \;\; and \;\; PUR_i < PUR_{\min} \qquad (5.14)$$

Mode 2 – collection of fractions with the required purity

$$C_{L\,i} \geq C_{\min} \;\; and \;\; PUR_i \geq PUR_{\min} \qquad (5.15)$$

Mode 3 - recycling the fraction with mixture of components

$$C_{L\,i} \geq C_{\min} \;\; and \;\; PUR_i < PUR_{\min} \qquad (5.16)$$

5.3.3 Numerical solution

The model equations are numerically solved using the software package PDECOL (Madsen and Sincovec, 1979). The calculation is based on the method of lines and orthogonal collocation in finite elements (OCFE) for discretization of the space variable. The partial differential equations (PDEs) are discretized in the axial direction using 20 finite elements and two interior collocation points in each element. The solver GEARIB (Hindmarsch, 1976) is used to integrate the system of ODEs in the time variable.

5.3.4 Performance criteria

The criteria used to determine the performance of this process are: purity (PURX), recovery (RCX), productivity (PRX) and solvent consumption (SCX). These criteria are based on mass unities, m_i and the formulas are shown in Table 5.1. Besides these four usual criteria, this study also includes the total solvent cost ($SC\$$), the energy cost for recovering the solvent by distillation ($EC\$$) and the product concentration of the components after separation (C_f).

It will be considered a mobile phase constituted by two solvents S1 and S2, in the volume proportion of $x_{S1} : x_{S2}$. The total solvent cost, $S\$$, is calculated as a weighted average of the prices of the solvents used as mobile phase:

Table 5.1 Performance criteria used in the optimization of the separation by preparative chromatography.

Performance criteria	Limonene
Purity (%)	$PURX_i = \frac{m_i}{\sum_{j=1}^{N} m_j} \times 100$
Recovery (%)	$RCX_i = \frac{m_i}{C_{0i} \times t_{inj} \times Q} \times 100$
Productivity (g*/h per l bed):	$PRX_i = \frac{m_i / t_c}{(1-\varepsilon)\ Vc}$
Solvent consumption (l/g*):	$SCX_i = \frac{tf_i \times Q}{m_i}$
Product concentration (g/l)	$C_{f\,i} = \frac{m_i}{tf_i \times Q}$
Solvent cost (EUR/g*)	$SC\$_i = SCX_i \times S\$$
Energy cost*** (EUR/g)	$EC\$_i = E \times SCX_i$

*g of product obtained: limonene or citral

variables: i – component, N – total number of components, m – mass of component (g), C_o – initial concentration (g/l), t_{inj} – injection time (min), Q – flow rate (ml/min), t_c - cycle time (min) , V_c – column volume (ml), tf – time of collection of fraction rich in component i (min).

$$S\$ = x_{S1} \times S1\$ + x_{S2} \times S2\$ \tag{5.17}$$

where x_{S1} is the volume fraction of solvent S1 in the mobile phase, x_{S2} is the volume fraction of solvent S1 in the mobile phase, $S1\$$ and $S2\$$ are the prices of solvent S1 and solvent S2, respectively.

The energy factor E used to calculate $EC\$$ represents the energy necessary to evaporate the solvent of the mobile phase and is given by:

$$E = x_{S1} \times \frac{\rho_1}{M_1}\left(Cp_{l_1}\left(T_{b_1} - 293\right) + \Delta H_{vap_1}\right) + x_{S2} \times \frac{\rho_2}{M_2}\left(Cp_{l_2}\left(T_{b_2} - 293\right) + \Delta H_{vap_2}\right) \tag{5.18}$$

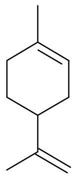
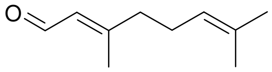
where ρ is the solvent density (kg/m3), M is molar mass (kg/mol), Cp_l is the liquid heat capacity (J/mol K), T_b is the boiling point (K) and ΔH_{vap} is the heat of vaporization (J/mol); the indexes 1 and 2 refer to the solvents S1 and S2 used as mobile phases S1/S2 $x_{S1} : x_{S2}$.

5.4 Case study: Fractionation of lemon oil

In this work, the deterpenation of essential oils by preparative HPLC is studied using the case study of separation of synthetic lemon oil, consisting in a binary mixture of limonene and citral. These two components are present in lemon oil and are representative of the two main fractions to separate: limonene represents the monoterpene hydrocarbon fraction to remove (non-polar), whereas citral represents the oxygenated fraction to concentrate (polar), containing the more important lemon fragrant components. The main properties of both components are presented in Table 5.2.

Lemon oil was selected for this study because it is one of the most used perfumery material to impart freshness and citric notes to the perfume composition. Moreover, it constitutes one of the natural raw materials that could be exploited in Portugal since the plant is well adapted to our territory.

Table 5.2 Physical-chemical properties of limonene and citral.

Chemical structure		
Compound	limonene	citral
Molecular formula	$C_{10}H_{16}$	$C_{10}H_{16}O$
Molar mass (g/mol)	136.24	152.24
Boiling point (°C)	175-176	229
Density (g/cm^3)	0.840	0.888
Odour description	mild citrus sweet orange lemon	lemon

5.5 Experimental

5.5.1 Materials

The following materials were used: (+)-limonene (98%, Fluka); citral (97%, Fluka); n-hexane (97%, Merck), n-heptane (99%, Merck), ethyl acetate (99.5%, Merck), ethanol (99.8%, Merck). The main characteristics of the solvents used (heptane, ethyl acetate, hexane, ethanol) are shown in Table 5.3.

5.5.2 Packing material

The adsorbent material used was Kromasil® Silica (Eka Nobel), with spherical particles of 10 µm diameter and pore size of 100 Å. The packing bed has 240 mm length and the interstitial porosity was determined as 0.43 and total porosity as 0.810 (Azevedo, 1994).

Table 5.3 Properties of solvents used as mobile phases or eluents.

Solvent	**Heptane**	**Ethyl acetate**	**Hexane**	**Ethanol**
Molecular formula	$C_7 H_{16}$	$CH_3 COOC_2 H_5$	$C_6 H_{14}$	$C_2 H_5 OH$
Molar mass (Kg/mol)¹	0.1002	0.0881	0.0862	0.0461
Density (Kg/m³)²	683.7	900.6	659.4	789.2
Viscosity (Pa·s)²	0.42×10^{-3}	0.45×10^{-3}	0.31×10^{-3}	1.1×10^{-3}
Boiling point (ºC)¹	98.4	77.1	69	78.3
Heat capacity (J/mol K)1	225	169.6	196	112
Heat of vaporization (J/mol) ¹	31.77×10^{3}	31.94×10^{3}	28.85×10^{3}	38.56×10^{3}
UV cutoff (nm)²	200	256	195	210
Polarity index²	0.1	4.4	0.1	-
Eluotropic value on silica²	0.01	0.48	0.01	-
Price (EUR/L)³	24.14	9.36	15.36	3.93

1 Source: NIST Chemistry WebBook (http://webbook.nist.gov/chemistry/)

2 Source: Burdick & Jackson solvents (http://www.bandj.com/BJProduct/Properties/SolventGroup.html)

3 Source: VWR Merck supply prices

5.5.3 Description of equipment

The separation was carried out in the preparative chromatographic equipment Prochrom (France) LC.60.VE.900.70, shown in Figure 5.3. The column is a stainless steel cylinder of 970 mm length and 60 mm internal diameter, with an internal piston actuated by a hydraulic jack that keep the packing material at constant pressure during operation - dynamic axial compression. The hydraulic system uses a high-pressure pneumatic pump, which sets the pressure in the piston equal to 10 times the pressure of the compressed air supplied to the pump. The packing bed used has a height of 240mm. The solvent (mobile phase) is delivered by a volume duplex diaphragm pump (Lewa, Germany), with operating range flow rate of 10-270 ml/min. The samples are injected using an auxiliary Lewa pump at 1 ml/s. The detector is a Spectra 100 UV-VIS, which is connected to a PC that acquires the absorbance during the experimental run. The equipment can be controlled manually or automatically by software.

5.5.4 Operating conditions

Two mobile phases were considered: n-heptane/ethyl acetate 85:15 and n-hexane/ethanol 85:15. The piston pressure was 30 bar. The UV absorbance was measured at the wavelengths 241 nm (n-heptane/ethyl acetate 85:15) and 220 nm (n-hexane/ethanol 85:15).

The equipment was operated in automatic mode, using a flow rate of 37 ml/min for all experiments. The room temperature was 25 °C.

Figure 5.3 Preparative HPLC equipment.

5.5.5 Samples

In each experiment, the solutions of limonene and citral were prepared using the same solvent of the mobile phase. The UV detector response is very different for the components limonene and citral, saturating very easily with citral and giving small response to limonene.

Two limonene solutions with concentration of 42 g/l and 28 g/l were prepared for both mobile phases. For citral, the concentration and injection volume had to be adjusted to avoid saturating UV detector, using solutions with concentration 14.9 g/l for mobile phase heptane/ethyl acetate 85:15, and 13.9 and 9.9 g/l for mobile phase hexane/ethanol 85:15.

5.6 Results

5.6.1 Determination of the isotherm parameters

Figure 5.4 and Figure 5.5 show the chromatograms obtained. Note that, although limonene is in higher amount in the binary mixture, its response is considerably lower than that for citral. Looking at the peak shape, it can be seen that they are almost symmetric. Therefore, it is considered that both components have a linear adsorption isotherm. In this case, the linear adsorption equilibrium constants can be determined by pulse experiments, as follows:

$$K_i = \frac{tr_i \times Q - \varepsilon \ V_c}{(1-\varepsilon) \ V_c} \qquad (5.19)$$

where K_i is the linear isotherm constant, tr_i is the retention time (min) of component i in the chromatographic column, Q is the flow rate (ml/min), ε is the bed porosity and V_c is the column volume (ml).

The values of the linear isotherm parameters are presented in Table 5.4 and Table 5.5.

Using heptane/ethyl acetate as mobile phase, citral yields a double peak, which corresponds to the two isomers neral and geranial. Collecting a fraction of each peak and analyzing by GC/MS confirmed this

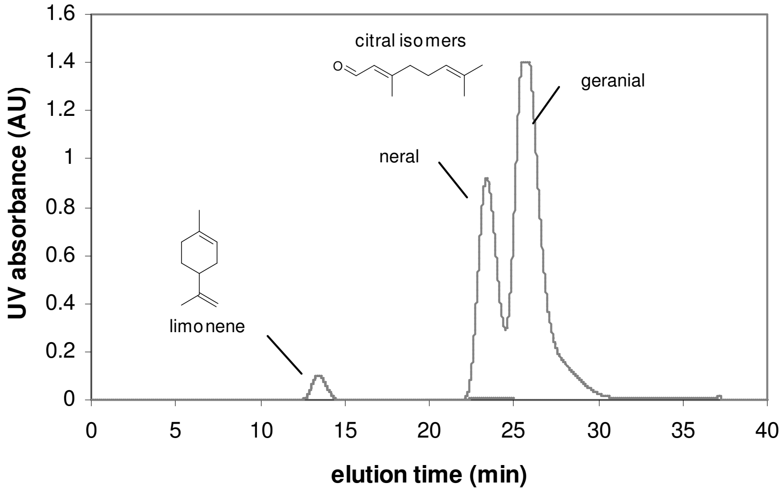


Figure 5.4 Separation of limonene/citral by preparative HPLC, using mobile phase heptane/ethyl acetate 85:15 and UV detection at 241 nm.

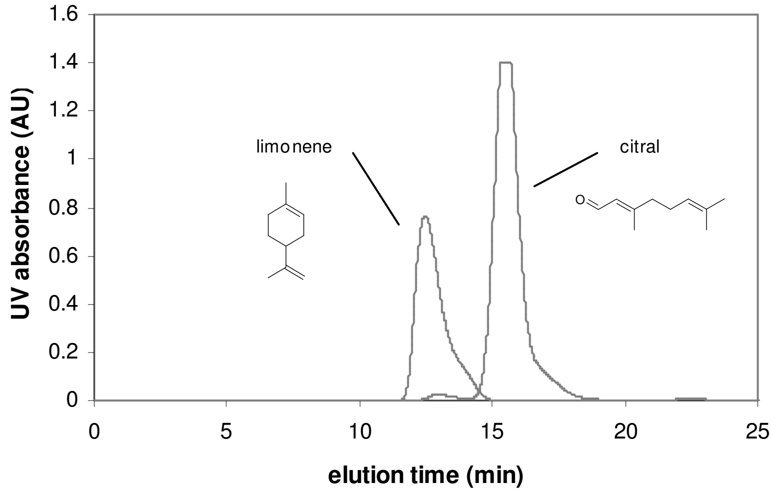


Figure 5.5 Separation of limonene/citral by preparative HPLC, using mobile phase hexane/ethanol 85:15,and UV detection at 220 nm.

Table 5.4 Linear isotherm parameters for mobile phase heptane/ethyl acetate 85:15.

Component	Retention time (min)	Isotherm constant
Limonene	13.2	0.5
citral: isomer neral	23.0	1.4
citral: isomer geranial	25.3	1.7

Table 5.5 Linear isotherm parameters for mobile phase hexane/ethanol 85:15.

Component	Retention time (min)	Isotherm constant
limonene	12.1	0.4
citral	15.0	0.7

fact. Moreover, the ratio of the two peaks is around 40:60, corresponding to the proportion of the two isomers in citral (chemical standard used in experiments) (B/R-Instrument-Corporation, 2004).

The separation of the citral isomers is little reported in the literature and only having an analysis purpose. Diagone et al. (2003) accomplish the separation by using cyclodextrin as a chiral additive in the mobile phase. Kikonyogo et al. (1999) used a reversed phase C18 column aqueous 0.1% trifluoroacetic acid/acetonitrile 3:1 to separate geranial and neral. B/R Instrument Corp (B/R-Instrument-Corporation, 2004) present an application note on the micro distillation apparatus, where a mixture of geranial/neral 62:38 is concentrated to a fraction of 96% geranial at 1 mmHg and 55 °C. However, citral is used as such (with both isomers) in the flavour and fragrance industry and as a starting material for the production of vitamins A and E, several carotenoids and other aroma chemicals. Taking this into account, the separation of the citral isomers will not be herein explored and they are collected together in the second fraction.

Although the UV detector response is very different for the components limonene and citral, it is possible to conclude that the components are well separated in time, using the mobile phase heptane/ethyl acetate 85:15.

In the case of the mobile phase hexane/ethanol 85:15, the double peak for citral does not occur. The retention times of the two components are closer than the previous mobile phase, but the peaks remain separated.

In summary, the two mobile phases tested provide the separation of the mixture limonene/citral, so both will be considered.

The simulator of preparative chromatography here described will be used next as a tool to select the best mobile phase and optimise the production of terpeneless lemon oil by preparative chromatography.

5.6.2 Optimisation of the separation of limonene/citral by simulation

The operating conditions, column dimensions and model parameters are shown in Table 5.6. The experimental time was corrected for the dead volume of tubing.

The experimental elution profiles are compared to the profiles obtained by simulation, in Figure 5.6 and Figure 5.7.

Table 5.6 Operating conditions, column dimensions and experimental parameters used in simulation of preparative chromatographic separation.

Column diameter, dc (mm)	60
Adsorption bed length, L (mm)	240
Flow rate, Q (ml/min)	37
Bed porosity, ε	0.435
Peclet number, Pe	4800
Initial concentration of limonene, Co(1) (g/L)	8.0
Initial concentration of citral, Co(2) (g/L)	2.0
Injection time, t inj (min)	1.0

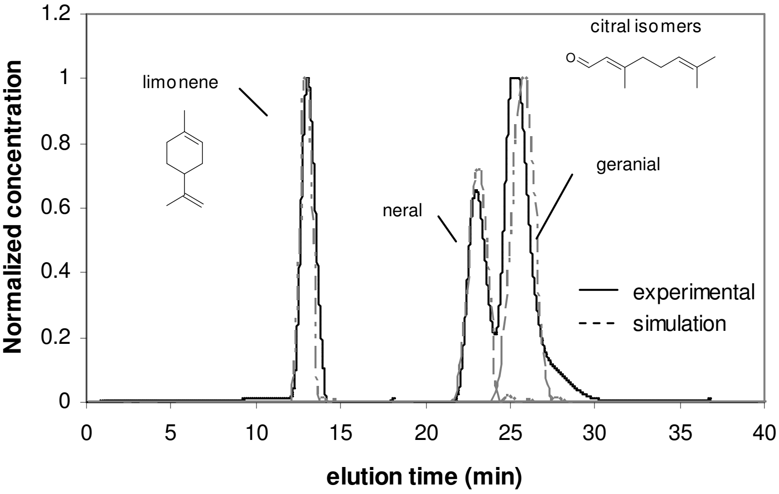


Figure 5.6 Comparison between concentration profiles obtained experimentally (continuous line) and by simulation (interrupted line) for mobile phase heptane/ethyl acetate 85:15.

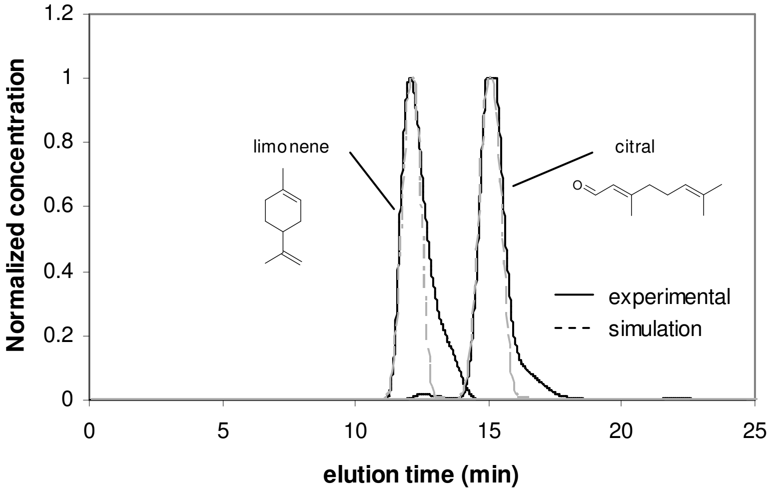


Figure 5.7 Comparison between concentration profiles obtained experimentally and (continuous line) and by simulation (interrupted line) for mobile phase hexane/ethanol 85:15.

Considering the standard elution mode, with one initial injection, both mobile phases studied accomplish the complete separation of limonene/citral, therefore purity and recovery are high (99-100%). Comparing the performance criteria shown in Table 5.7, the mobile phase hexane/ethanol 85: 15 is more advantageous in all aspects, presenting a productivity 1.7 times higher and half of the solvent consumption. As the product is more concentrated in citral, there is a decrease of solvent evaporation costs (EC$) and the price is also smaller.

Although the purity and recovery of the components are already high, the productivity and solvent consumption may be improved. Considering that the components limonene and citral are well separated, the recycling mode is not applicable in this case.

The optimisation is based on two different operating modes so that there is a continuous production of both components, obtaining alternatively limonene and citral as products. These modes are:

(A) Consecutive injections with volume overload at regular intervals

Mode applied to mobile phase heptane/ethyl acetate 85:15. As the 2 components are well separated in time, it is possible to use a long injection time (volume overload) of 8.1 min. To increase productivity, consecutive injections in intervals of 23.2 min were made (Figure 5.8).

(B) Consecutive pulse injections at regular intervals

Mode applied to mobile phase hexane/ethanol 85:15. Considering that the 2 components are separated as neighbouring peaks, pulse injections with injection time of 1 min are made. To increase productivity many successive injections were made, in intervals of 5.5 min (Figure 5.9).

The calculated performance parameters are shown in Table 5.8. Now the situation is reversed: using the mobile phase heptane/ethyl acetate 85:15 the productivity is increased 10 times and is double of the one obtained with the mobile phase hexane/ethanol 85:15. The solvent consumption and energy costs for solvent recovery are reduced 68%. When comparing both mobile phases, heptane/ethyl acetate 85:15 led to 1.5 times less of solvent consumption and energy cost. Therefore the mobile phase heptane/ethyl acetate 85:15 is chosen to perform the separation of limonene and citral.

Table 5.7 Performance parameters obtained in simple elution mode with one injection.

	heptane/ethyl acetate 85:15		**hexane/ethanol 85:15**	
	limonene	citral	limonene	citral
PUR (%)	100.00	100.00	100.00	100.00
RCX (%)	99.39	99.32	99.52	99.36
PRX (g/h per l bed)	1.70	0.42	2.85	0.71
SCX (l/g)	0.24	2.47	0.24	1.06
Cs (g/l)	4.18	0.41	4.98	1.10
SC$ (EUR/g*)	5.24	54.08	3.26	14.42
EC$ (EUR/g*)	0.002	0.023	0.002	0.009

*g of product obtained: limonene or citral

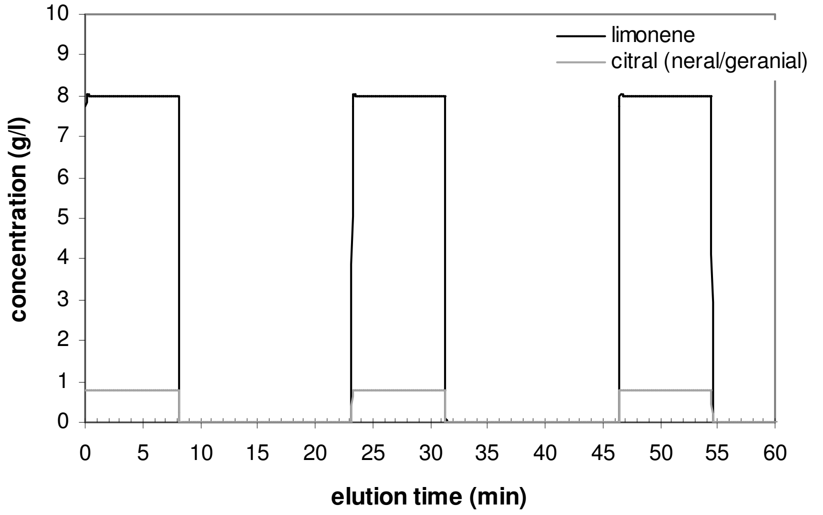
Table 5.8 Performance parameters obtained in optimised mode for the separation of the solution of limonene/citral, using: (1) mobile phase heptane/ethyl acetate 85:15 and optimised mode (A); (2) mobile phase hexane/ethanol 85:15 and optimised mode (B).

	(1) heptane/ethyl acetate 85:15 optimised mode (A)		**(2) hexane/ethanol 85:15 optimised mode (B)**	
	limonene	citral	limonene	citral
PUR (%)	100.00	100.00	100.00	100.00
RCX (%)	99.92	99.63	99.52	99.36
PRX (g/h per l bed)	17.36	4.33	9.51	2.37
SCX (l/g)	0.14	0.72	0.24	1.06
Cs (g/l)	7.15	1.39	4.19	0.95
SC$ (EUR/g*)	3.06	15.76	3.26	14.42
EC$ (EUR/g*)	0.001	0.007	0.0023	0.0104

*g of product obtained: limonene or citral

(a)

Concentration profile: inlet of column



(b)

Concentration profile: outlet of column

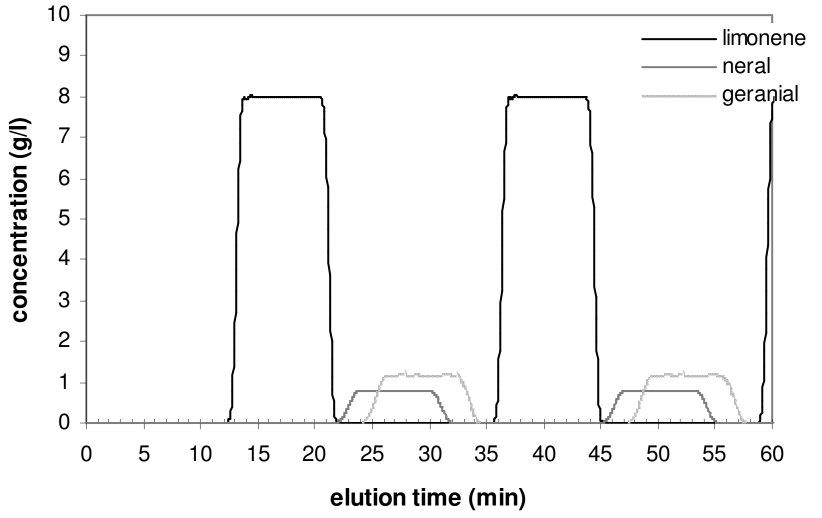
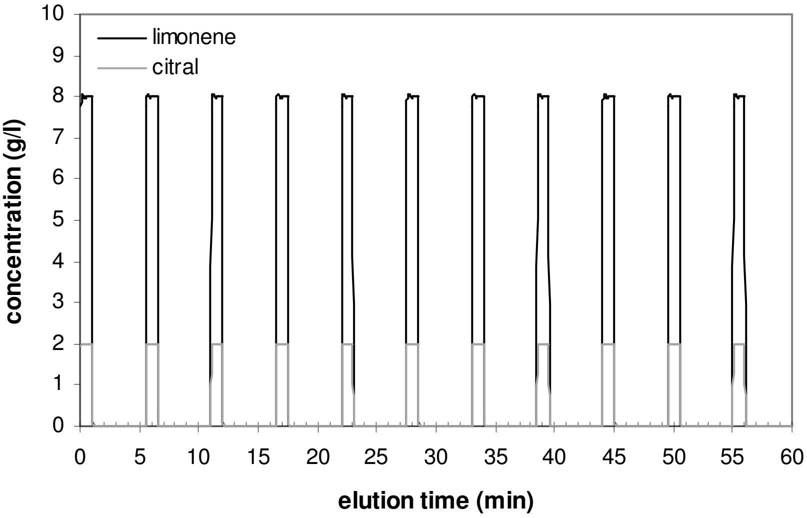


Figure 5.8 Simulation of separation of limonene/citral by preparative HPLC, using mobile phase heptane/ethyl acetate 85:15 at optimised conditions (A): (a) concentration profile at inlet of chromatographic column and (b) concentration profile at outlet of chromatographic column. The two peaks observed correspond to the citral isomers, geranial and neral.

(a)

Concentration profile: inlet of column



(b)

Concentration profile: outlet of column

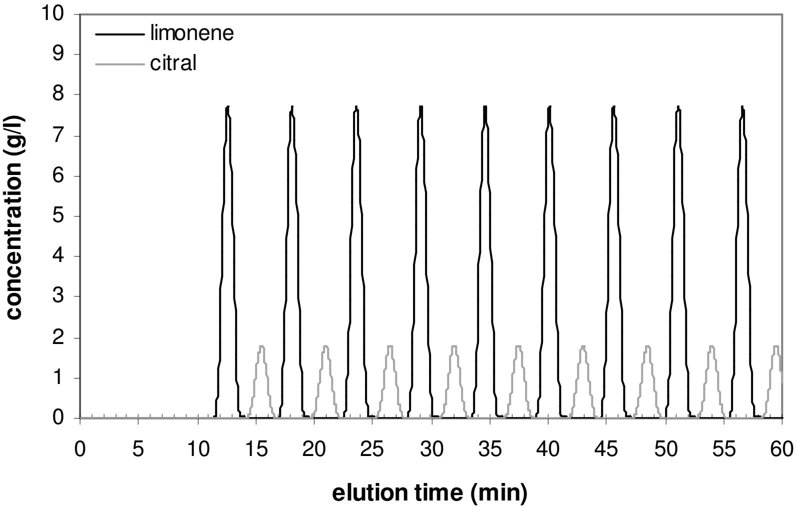


Figure 5.9 Simulation of separation of limonene/citral by preparative HPLC, using mobile phase hexane/ethanol 85:15 at optimised conditions (B): (a) concentration profile at inlet of chromatographic column and (b) concentration profile at outlet of chromatographic column.

5.7 Conclusion

The methodology herein developed enables the optimisation of the separation conditions and helps in the selection of the mobile phase that will provide effective separation and better productivity and economy in deterpenation of citrus essential oils using silica as adsorbent.

A case study of separation of the binary model mixture limonene/citral as synthetic lemon oil was used to simulate and optimise the separation. There were found two mobile-phases that accomplish the separation of the two components: heptane/ethyl acetate 85:15 and hexane/ethanol 85:15. Comparing the performance for standard elution, the mobile phase with the best performance is hexane/ethanol 85:15, since the elution time is considerably lower than the mobile phase heptane/ethyl acetate. However, in optimized operating conditions, this situation is reversed and the mobile phase that leads to best productivity and least solvent consumption is heptane/ethyl acetate 85:15. Therefore the choice for mobile phase for the limonene/citral separation is heptane/ethyl acetate 85:15.

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CHAPTER 6

PERFUME DESIGN WITH SCIENTIFIC BASIS

In this work, the innovative concept of Perfumery Ternary Diagram (PTD) is introduced, combining the Perfume Pyramid Structure with Engineering Ternary Diagrams (Mata *et al.*, *2004; 2005a; b; c*). These diagrams allow a fast theoretical evaluation of the odour value in the headspace for all possible concentrations of a perfume liquid mixture constituted by three fragrant components and one or more solvents, eliminating this way some trial and error experimental work to analyze the headspace composition. The effects of the non-idealities in the liquid phase due to molecular interactions, amount of solvent ethanol and adding another fragrant note were analyzed based on a first test mixture constituted by limonene, geraniol, vanillin and ethanol. Afterwards, four other systems were tested, namely: (S1) benzaldehyde, 2-phenylethanol, eugenol, ethanol; (S2) α-pinene, linalool, vanillin, ethanol; (S3) limonene, geraniol, tonalid, ethanol; and (S4) limonene, linalool, geraniol, ethanol.

6.1 Introduction: odour value concept

The odour intensity is expressed in terms of a parameter called odour value (OV) that is expressed as the ratio of headspace concentration (in the gas phase) and odour threshold value (the minimum concentration in the gas phase to be detected by the olfactory sense) (Calkin and Jellinek, 1994):

$$OV_i = \frac{C_{hs_i}}{C_{thr_i}} \tag{6.1}$$

where OV is odour value, C_{hs_i} is headspace concentration (g/m^3) and C_{thr_i} is the odour threshold value in air (g/m^3).

The OV is related with odour intensity and must be higher than one in order to perceive the fragrant component. However, these OV values are not absolute measurements for intensity since the olfactory response is not linear, depending on the nature of the odour component and saturating the nose when the concentrations are very high. Therefore odour values should be considered in relative terms and as a rather good prediction of odour intensity, in the same way that the vapour pressure is used to characterize volatility. It is important to notice that odour intensity is not additive. So, the odour intensity of a mixture is related with the component having the highest odour intensity or odour value (Appell, 1969).

Perfumery materials with the higher values of OV are preferred, especially for creating formulations with the best cost effectiveness: getting the same odour effect at the lowest cost, calculated as cost per unit of odour value.

The perfume system is considered as consisting in a complex fragrant liquid mixture and a corresponding air phase above it, the headspace, which we smell, as it is illustrated in Figure 6.1. In this work, the following assumptions were made:

(i) The headspace is an ideal mixture, since the components are highly diluted:

$$C_{hs_i} = \frac{y_i M_i P}{RT} \tag{6.2}$$

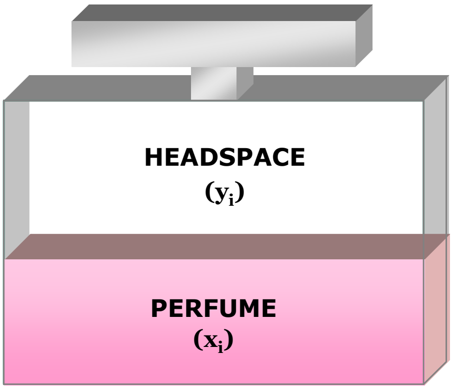


Figure 6.1 Perfume system composed of perfume liquid phase and vapour phase (headspace).

where C_{hs_i} is the concentration of component i in the headspace (g/m³), y_i is the gas phase molar fraction of component i, P is the total gas phase pressure (bar), M_i is the molar mass of i (g/mol), R is the ideal gas constant (J mol^{-1} K^{-1})and T is the absolute temperature (K).

(ii) The liquid is considered as a non-ideal solution, because its molecules have different intermolecular forces, and the headspace is in equilibrium with the liquid perfume mixture:

$$y_i = \gamma_i x_i \frac{P_i^{sat}}{P} \tag{6.3}$$

where y_i is the gas phase molar fraction of component i in the headspace in equilibrium with the liquid with composition x_i, γ_i is the activity coefficient of i in the liquid and P_i^{sat} is the saturated vapour pressure of component i (bar). In this work, the values of γ_i were calculated using the UNIFAC method, which is based on molecular group contribution (Reid *et al.*, 1987). This method takes into account the contribution due to differences in molecular size and molecular shape – combinatorial part - and the contribution due to molecular interactions – residual part. A detailed description of the UNIFAC method is shown in Appendix A.

Combining equations 6.1 to 6.3, OV is expressed in function of the liquid composition fraction:

$$OV_i = \gamma_i x_i \left(\frac{P_i^{sat} M_i}{C_{thr_i}} \right) \left(\frac{1}{RT} \right) \tag{6.4}$$

This equation relates the liquid perfume composition, x_i with the human sensory response of the evaporated perfume.

In this model, it is considered that the perfume mixture smells more strongly to the component present in the headspace above it with the highest OV. For example, a typical perfume has a highly volatile top note, which is noticeable when smelling the perfume bottle or until some minutes after application. It means that the odour value of the top note is higher than that of the middle and base notes. Middle note is normally only noticeable after application and after the top note has disappeared, i.e, due to the decrease in the concentration of the top note, the odour value of the middle note gets higher than that of the top note. Another special rule that a good perfume should follow is that the alcohol should never have the highest odour value, in order to not be perceived and rejected by the consumer.

Therefore, for a perfume mixture of N components, its characteristic odour value, OV_{perf} is related with the component with the highest odour value, OV_{MAX} :

$$OV_{perf} = OV_{MAX} = MAX\left(OV_A,\ OV_B, \ldots, OV_N\right) \tag{6.5}$$

The activity coefficient γ_i of a component i in a mixture provides a useful way of accessing the affinity of this odourant i to the surrounding medium (Behan and Perring, 1987). For $\gamma_i > 1$, the partial pressure of component i is higher than that predicted for an ideal solution, indicating that this volatile has no affinity to the medium and so it will be 'pushed-out' of the liquid system into the gas phase. On the contrary, for $\gamma_i < 1$, implies that the component i has a high affinity to the medium, so its partial pressure will be lower, meaning that there is less material in the gas phase than expected if we consider the liquid an ideal solution and so there occurs an increased retention in the liquid.

As a consequence, the odour value can change using different solvents or different quantities of each fragrant chemical for a specific multi-component mixture. So, the perfumer needs to know how to increase the performance of selected materials for a given application, by choosing the right composition.

6.1.1 Objectives of this work

In this work, a new methodology based on perfumery ternary diagram (PTD) (Mata *et al.*, *2004; 2005a; b; c*) was developed to help perfumers in the prediction of the headspace odour value (OV) for a ternary perfume mixture diluted or not in ethanol was used. This technique allows a fast headspace evaluation for all liquid combinations of three fragrant components and for a fixed amount of one or more solvents. The effect of non-ideality in the liquid perfume phase due to intermolecular interactions is studied and compared with the ideal liquid perfume mixture assumption. The values of vapour pressure and odour thresholds used in the calculation were taken from the literature (Calkin and Jellinek, 1994).

6.2 Case study: simple binary perfume mixture

Consider a binary liquid mixture, **A+C**, constituted by two fragrant chemical components having different volatilities and polarities, where **A** represents a non-polar top note and **C** a polar base note. In this example, we will consider that component A is limonene and component C is vanillin.

For each mixture, there exist n_A moles of component A and n_C moles of component C. Their molar fractions x_A and x_C, respectively, are given by:

$$x_A = \frac{n_A}{n_A + n_C} \tag{6.6}$$

$$x_C = \frac{n_C}{n_A + n_C} \tag{6.7}$$

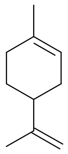
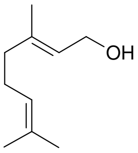
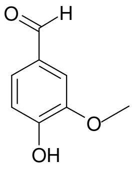
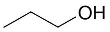
As it is a binary misture:

$$x_A + x_C = 1 \tag{6.8}$$

For each mixture (x_A, x_C) the odour values of each component, OV_A and OV_C are calculated using Equation 6.4. The physical parameters of each component are shown in Table 6.1.

Figure 6.2-a shows the odour value for each component as a function of liquid composition in terms of

Table 6.1 Physical constants and odour description of components used in the perfume test mixture, at 25 ºC.

.	**Component**	**Molecular formula**	**M** (g/mol)	C_{thr_i} (1) (g/m3)	P_i^{sat} (1) (Pa)	**Odour description**
A	limonene	$C_{10}H_{16}$ 	136.1	2.45×10^{-3}	2.05×10^{2}	lemon citric fresh
B	geraniol	$C_{10}H_{18}O$ 	154.3	2.48×10^{-5}	2.67×10^{0}	rose floral
C	vanillin	$C_8H_8O_3$ 	152.2	1.87×10^{-7}	1.60×10^{-2}	vanilla sweet warm
S	ethanol	C_2H_6O 	46.0	5.53×10^{-2}	7.27×10^{3}	alcohol pungent

(1) data from Calkin and Jellinek (1994)

molar fraction of C, x_C =(1-x_A), for all the possible combinations A+C. It can be seen that at high amounts of A, $x_A > 0.5$, it is C that has the highest OV, $OV_{MAX} = OV_C$, and when x_C >0.5, $OV_{MAX} = OV_A$. This case shows clearly how the liquid and the headspace composition can be completely different, for example, preparing a perfume mixture with 80% of limonene, it will smell more strongly to vanillin and not limonene. The reason for this is that as A and C have very different polarities, when A is in minor quantity, its molecules are not able to break the strong polar intermolecular bonds between molecules of C to mix with them and so they are 'pushed out' from the

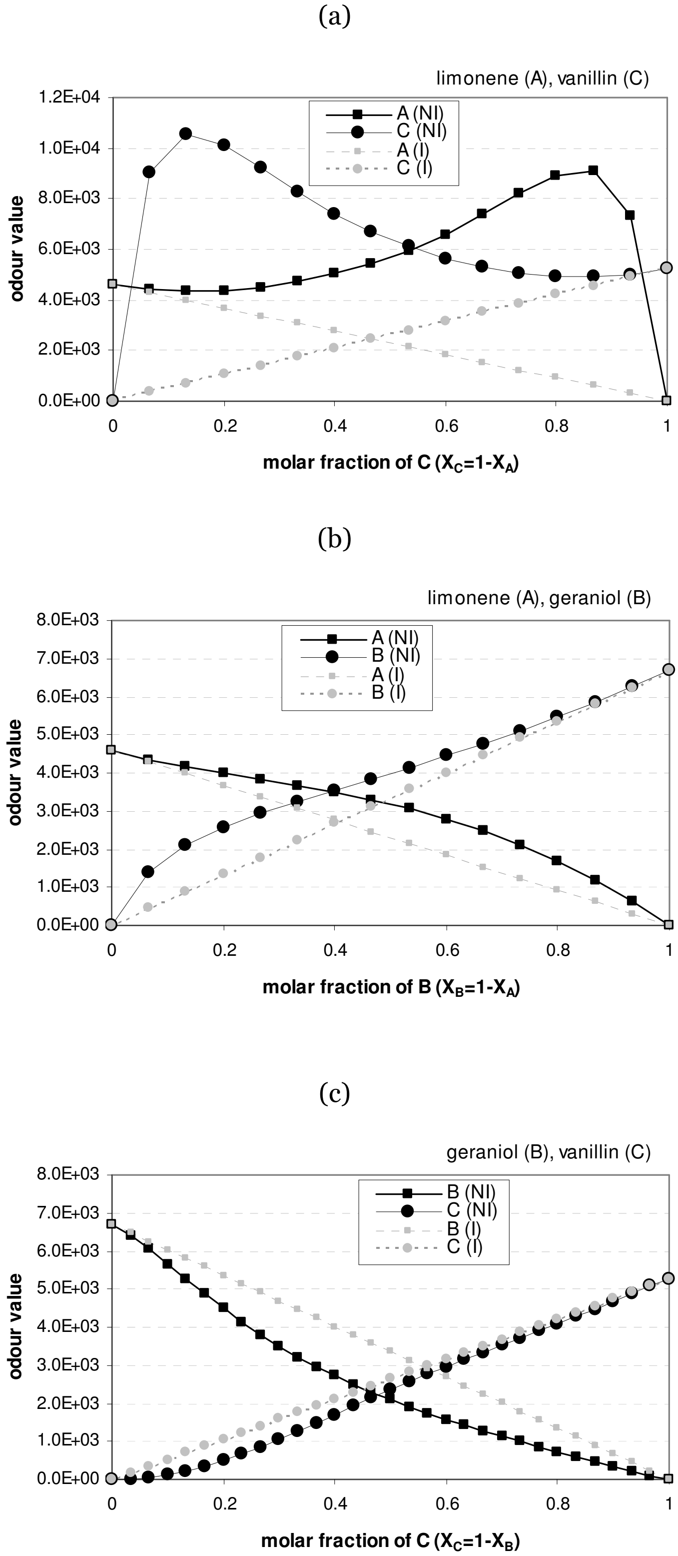


Figure 6.2 Comparison of odour value for the binary mixtures composed of limonene (A), geraniol (B) and vanillin (C), considering the ideal case (I) and non-ideal case (NI): (a) A+C; (b) B+C; (c) A+B.

solution to the headspace by the bulk molecules of C. Therefore, the headspace concentration of A is higher and consequently also its odour value increases. The reverse happens when the bulk is constituted by molecules of A.

In the same plot it can be seen also the comparison between these odour values obtained for non-ideal mixtures (NI), when $\gamma_i \neq 1$ and with that obtained for ideal mixtures (I), when $\gamma_i = 1$ in Equation 6.4.

In the non-ideal case, the biggest deviations of OV_A occur for high values of x_C and the biggest deviations of OV_C occur for high values of x_A. This behaviour is again explained by the polarity differences between molecules A and C, where their high non-affinity causes a higher evaporation than expected for the component in smaller quantity and therefore there are higher deviations from ideality.

Lower differences between the molecules polarity results in an approximation to the ideality as can be seen in Figure 6.2-b and c. In this system of geraniol and vanillin, B+C, they have higher affinity to the surrounding media because both are polar, increasing their retention in the media and lowering this way their values of γ_i. Consequently, the headspace concentration is smaller and the OV values are smaller than the OV values obtained when considering the mixture as an ideal solution.

The binary mixture limonene and geraniol, A+B, has a behaviour intermediate between the two cases mentioned A+C and B+C. The reason is that the difference in polarity between limonene (non-polar) and geraniol (moderately polar) is not as high as in the case of the mixture A+C. Nevertheless, the predicted OV values considering the non-ideal case are higher than OV values obtained for the ideal case.

6.2.1 Effect of the solvent on the OV

Figure 6.3 shows the effect of the solvent (S), in the odour value of each component of the perfume mixture limonene and vanillin, A+C. The solvent used is ethanol, the most common in perfumery. The molar fractions x_A’ and x_B’ are represented in a basis without solvent S, calculated using Equation 6.6 and Equation 6.7. As ethanol is a polar solvent, the non-polar component (A) will decrease its affinity to the medium with increasing molar fraction of solvent. Therefore, adding a small amount of S,

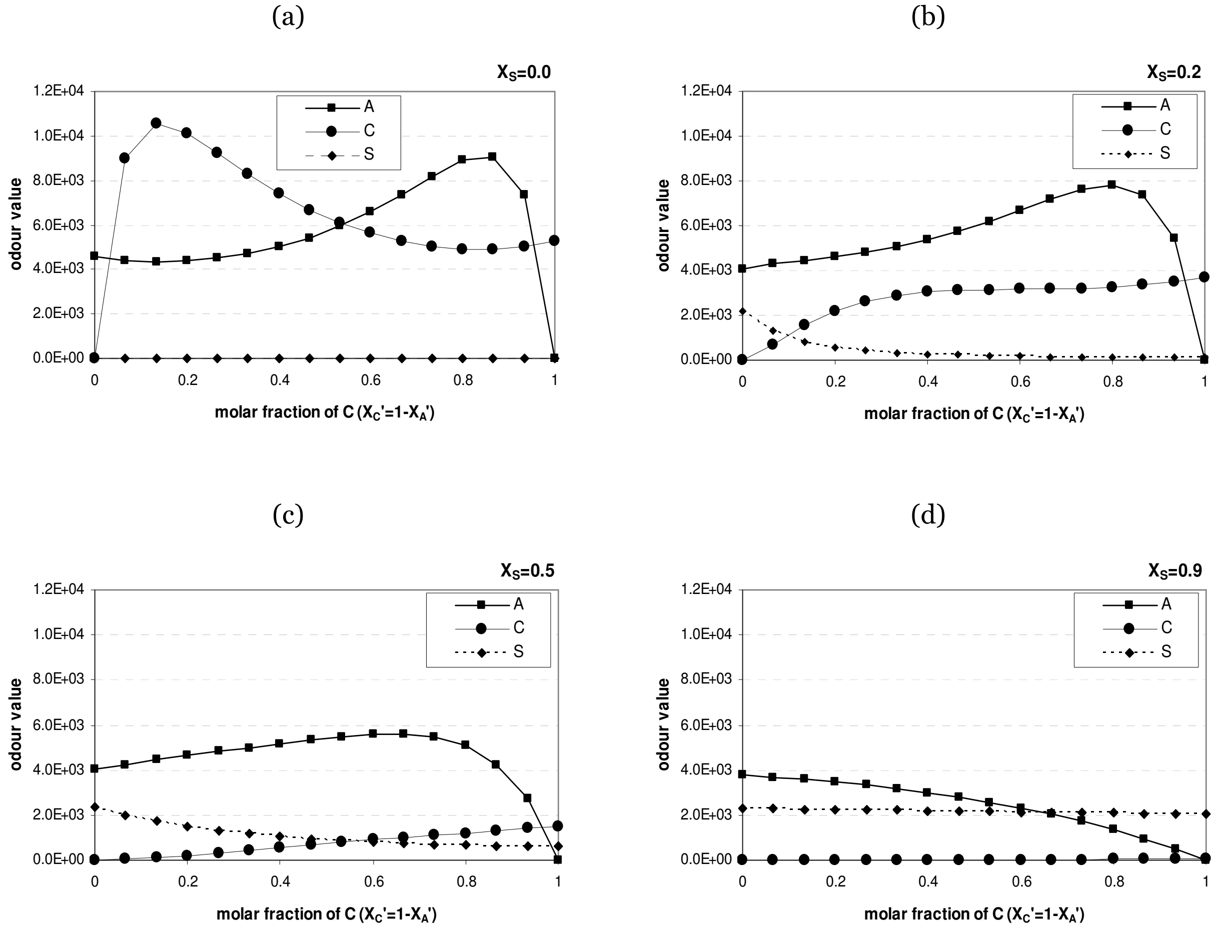


Figure 6.3 Effect of solvent (S) in the odour value of a binary mixture of limonene (A) and vanillin (C): (a) Xs=0.0; (b) Xs=0.2; (c) Xs=0.5 and (d) Xs=0.9.

$x_S = 0.2$, is enough to change the odour value profile, as can be seen in Figure 6.3 – b. Limonene is then the component with the highest OV, in almost the whole range of concentrations. However, at higher molar fraction of the solvent, there is a point when $OV_{MAX} = OV_S$, as is the case shown for $x_S = 0.9$. In this case, to make a mixture for which the highest OV is that of limonene, there is a minimum amount that should be used, $x'_A > 0.3$, so that $OV_{MAX} = OV_A$. Note that for this diluted perfume mixture, vanillin has never the highest odour value.

Considering now the perfume mixture limonene and geraniol A+B, the effect of ethanol is less pronounced, since the polarity difference between A and B is smaller, as is shown in Figure 6.4. In this

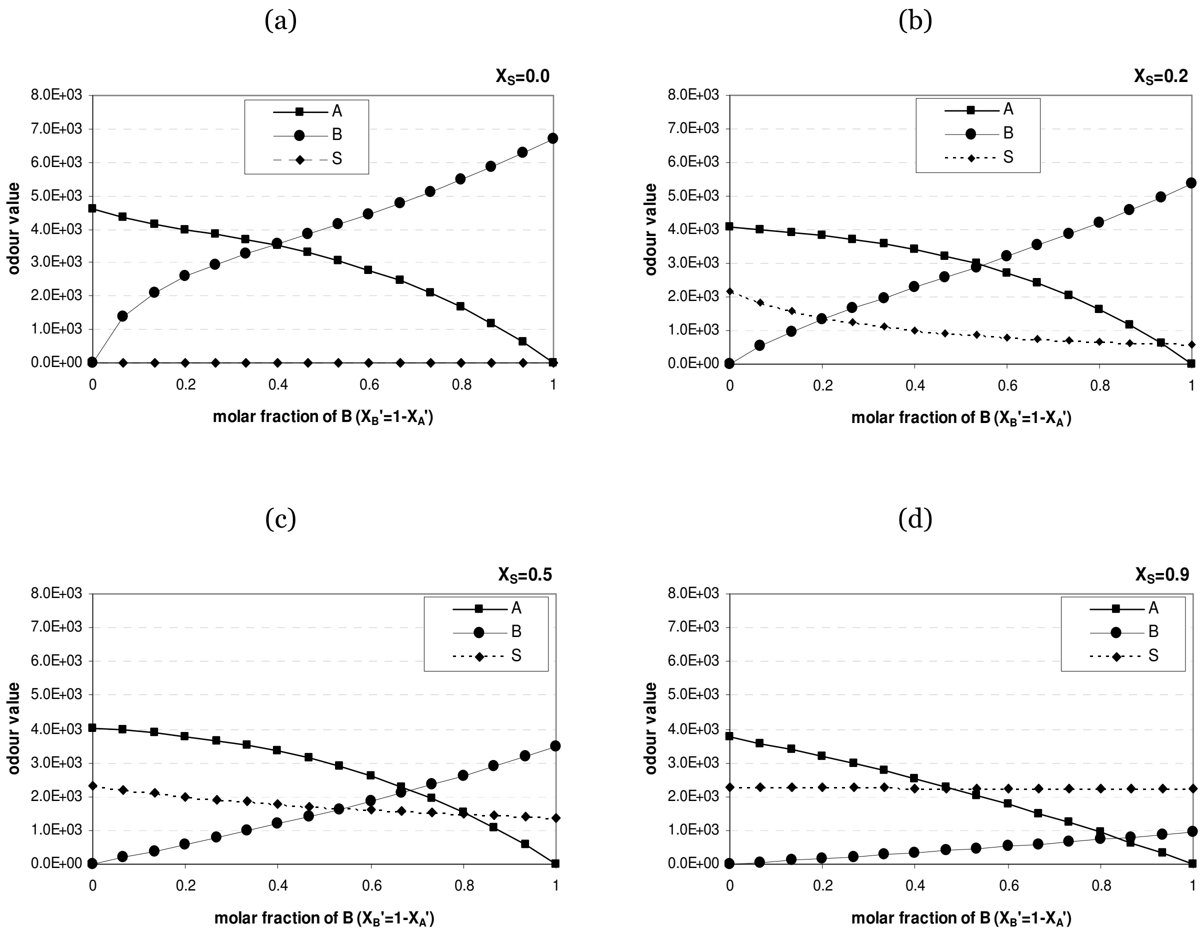


Figure 6.4 Effect of solvent (S) in the odour value of a binary mixture of limonene (A) and geraniol (B): (a) Xs=0.0; (b) Xs=0.2; (c) Xs=0.5 and (d) Xs=0.9.

case, at $x_S = 0$, for limonene to have the highest OV, there must be $x'_A > 0.6$. Increasing the molar fraction to $x_S = 0.2$, the amount of limonene necessary for this effect decreases to $x'_A > 0.55$ and then to $x'_A > 0.3$ for $x_S = 0.5$. Otherwise, it is geraniol that has the maximum OV. At very diluted perfume mixture, $x_S = 0.9$, the necessary amount of A increase again, $x'_A > 0.5$ so that the maximum OV is that of A and not of S. Note that also in this case, geraniol has never the highest odour value.

6.2.2 Effect of adding another fragrant note on the OV

In Figure 6.5 is shown the effect on odour value of adding a middle polar note, geraniol, B, to a mixture of limonene and vanillin, A+C. It can be seen that at $x_B = 0.1$, B equilibrates the odour values of A and C. However, as the amount of B increases, the medium becomes more polar and therefore A is ‘pushed out’ from the solution, leading to $OV_{MAX} = OV_A$ over a wider range of concentrations.

Figure 6.6 shows the effect of adding one base note – or fixative - to the mixture of limonene+geraniol, A+B. The base note is vanillin, C. It can be seen that an increase in C leads to an increase in OV_A and in a decrease in OV_B. The reason is because vanillin is a polar molecule and as its concentration

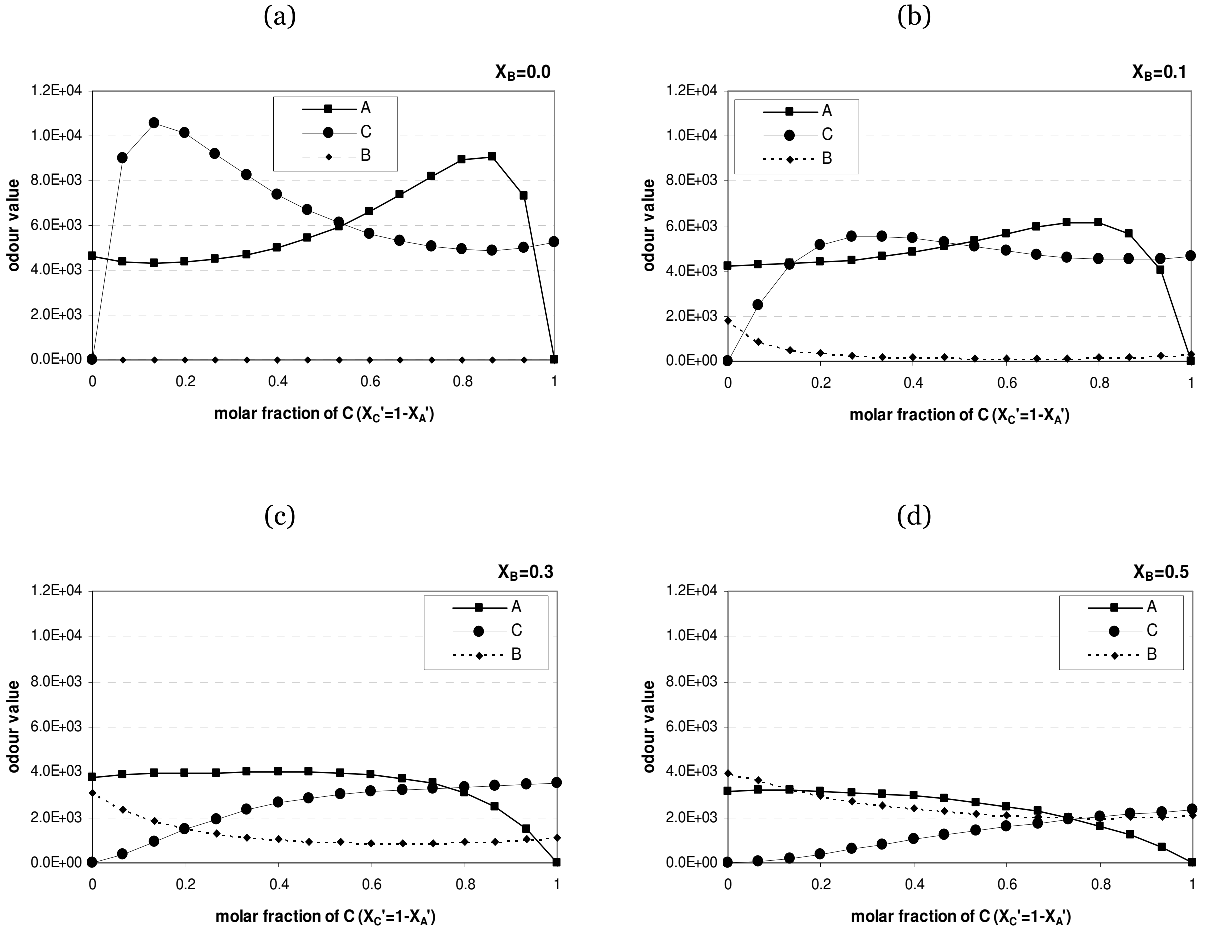

(a) (b) (c) (d)

Figure 6.5 Effect of middle note geraniol (B) in the odour value of a binary mixture of limonene (A) and vanillin (C): (a) XB=0.0; (b) XB=0.1; (c) XB=0.3 and (d)) XB=0.5.

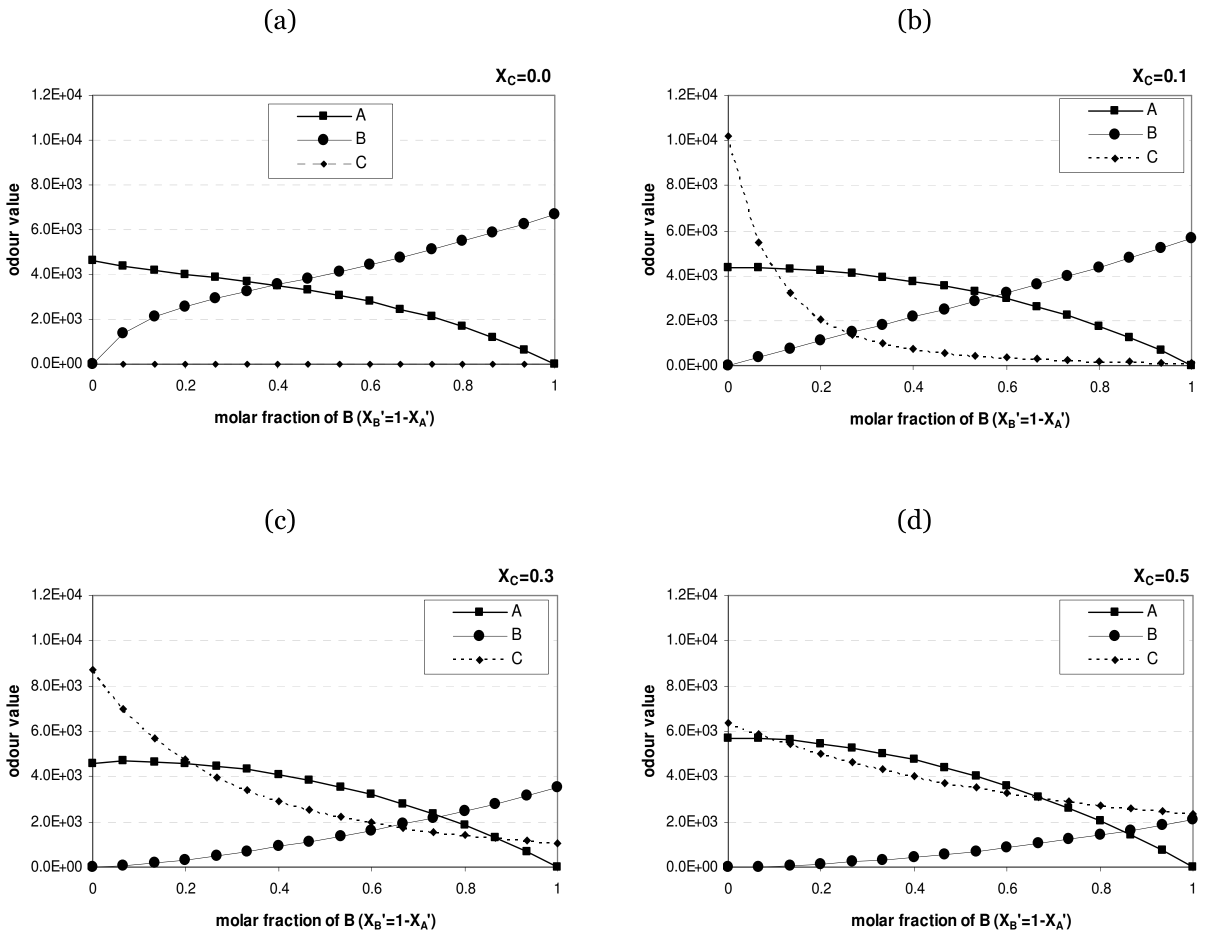

(a) (b) (c) (d)

Figure 6.6 Effect of base note vanillin (C) in the odour value of a binary mixture of limonene (A) and geraniol (B): (a) $X_C=0.0$*; (b)* $X_C=0.1$*; (c)* $X_C=0.3$ *and (d))* $X_C=0.5$*.*

increases, the media becomes more polar and so it tends to 'push out' the molecules of the non-polar component, limonene (A) from the liquid phase and, at the same time, to retain the polar component, geraniol (B).

Perfumers may use materials of low volatility in their perfumes intending thereby to slow down the evaporation of more volatile perfume components. This is the practice commonly referred to as fixation. Strong mutual attraction between solvent and perfume material results in a decreased tendency of the perfume material to evaporate, and hence lowers the odour intensity of that material above the solution. Since perfume compositions are blends of perfume materials that differ widely in their polarities (even single essential oils represent such blends), the different components of a perfume are held back to differing degrees when the perfume is dissolved in a solvent. The patterns of differential holding vary greatly between different solvent systems. As a result, if a given perfume composition is dissolved in two different systems, the odour of their headspace will be distinctly different. It can therefore be stated that the odour of any perfumed product is affected by the product base in two ways: by the odour of the base itself and by the way the base, through physical attraction forces, affects the odour of the perfume (Behan and Perring, 1987).

In conclusion, the choice of the fixative is very important because it will determine both (i) the characteristic smell of the perfume and (ii) its persistence.

6.3 Ternary perfume mixtures

Now, let us consider a ternary liquid mixture, **A+B+C**, constituted by three chemical components having different volatilities, each one representing one type of fragrant notes: **A** is a top note, **B** a middle note and **C** a base note. In some cases, it is also considered that a certain amount of solvent, **S**, in this case ethanol is added to the mixture, resulting this way in a quaternary mixture, that will be called test perfume.

The amount of each component in the test perfume is given by its molar fraction: x_A, x_B, x_C and x_S and we will call this mixture X.

To simplify, this quaternary system is transformed into a ternary system, by recalculating the molar fractions for A, B and C in a solvent free basis, resulting in the new fractions:

$$x_A' = \frac{x_A}{x_A + x_B + x_C} \tag{6.9}$$

$$x_B' = \frac{x_B}{x_A + x_B + x_C} \tag{6.10}$$

$$x_C' = \frac{x_C}{x_A + x_B + x_C} \tag{6.11}$$

These three quantities are then represented in a ternary diagram (Ruthven, 1997) as it is shown in Figure 6.7.

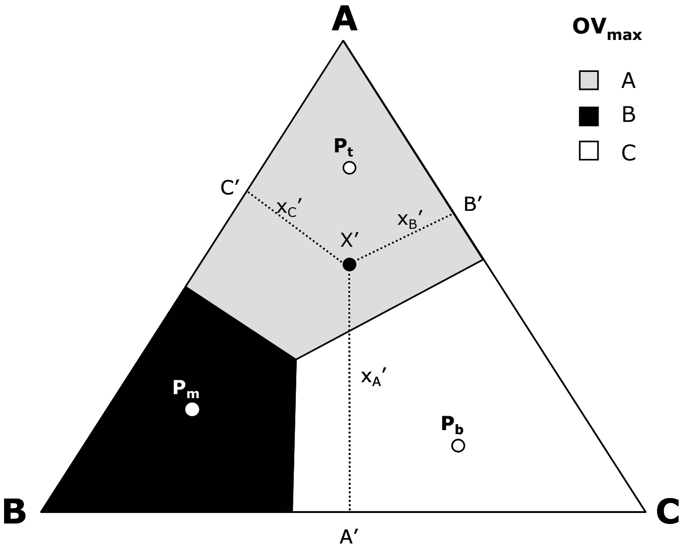


Figure 6.7 Scheme of Perfumery Ternary Diagram (PTD): representation of general ternary mixture with components A, B and C, with composition X' and areas with different maximum odour value.

Considering a general liquid mixture, which composition is represented by point X', the composition (solvent-free basis) of component A, x_A', is given by the distance $\overline{X'A'}$, the composition of B, x_B', is given by the distance $\overline{X'B'}$ and the composition of C, x_C', is given by the distance $\overline{X'C'}$. It is considered that the mole fraction of solvent, x_S, is known and constant for each ternary diagram. As it can be seen, the ternary diagram represents all liquid compositions that could be made experimentally using different quantities of A, B and C, for a given amount of ethanol, S.

6.3.1 Perfumery ternary diagram (PTD) concept

Now, for each liquid perfume mixture, X' (i.e., each point in the ternary diagram) the odour value of each component in that mixture, OV_i, is calculated using Equation 6.4. After the calculation of all the individual odour values, for a given mixture X' (represented in the ternary diagram) the maximum odour value is determined:

$$OV_{MAX} = MAX(OV_A, OV_B, OV_C, OV_S), \textit{for point } X' \qquad (6.12)$$

After the determination of the component that has the $OV_{\max}$ for a large number of points of the ternary diagram, we obtain as a result the *Perfumery Ternary Diagram* (PTD), as shown in Figure 6.7. In this diagram three different areas can be distinguished, giving some highlight to the following typical composition points: P_t with $OV_{MAX} = OV_A$ (grey area); P_m with $OV_{MAX} = OV_B$ (black area) and P_b with $OV_{MAX} = OV_C$ (white area), being the subscripts: t –top note, m-middle note and b-base note.

There can exist also points for which solvent has the highest odour value: $OV_{MAX} = OV_S$. These points are referred as P_s. This situation happens generally for mixtures having a high content of solvent, as it will be seen below.

6.3.2 Perfume test case

The developed *Perfumery Ternary Diagram* (PTD) strategy was tested using the system: limonene (A), geraniol (B) and vanillin (C). Again, A represents a top note, B a middle note and C a base note or fixative. In this first example, the presence of ethanol is not considered, meaning that $x_i^{'} = x_i$, $i = A, B, C$. This mixture can be a representation of a perfume concentrate or a perfume some seconds after application, the time that is needed to evaporate the ethanol.

Table 6.1 shows the properties for each component that are used for the calculation of each odour value, OV, at 25°C (298.15 K), namely molecular weight, saturated vapour pressure and odour threshold in air. At this point, the values of saturated vapour pressure and odour threshold in air used for this study were found in the literature. However, it is important to refer that the values found in the literature vary considerably, especially the odour threshold values, sometimes in several orders of magnitude, depending on the method and experimental conditions at which they were determined and that many times are not referred.

Taking into account that the sensibility to odours varies from person to person, this fact must be considered when a PTD is determined, i.e., the odour threshold values ($C_{thr\,i}$) should be determined by the perfumer/s that will make and test the perfume mixture composition, chosen from the PTD. Therefore, later in this work these values will be determined experimentally so that we can build our own database.

6.3.3 Effect of the liquid non-ideality on the PTD

Figure 6.8 shows the resulting PTD for the ternary system chosen. Each point in the diagram represents one different liquid composition of mixture A+B+C and, by the different type of point represented, it can be seen which is the component having the highest odour value, OV_{MAX}. It also shows the differences due the effect of the non-idealities in the liquid mixture, once in the construction of these two PTDs, the values of OV_i for each point where calculated (Equation 6.4) assuming that: (a) the liquid solution is ideal, with $\gamma_i = 1$; and (b) the liquid solution is non-ideal with $\gamma_i \neq 1$.

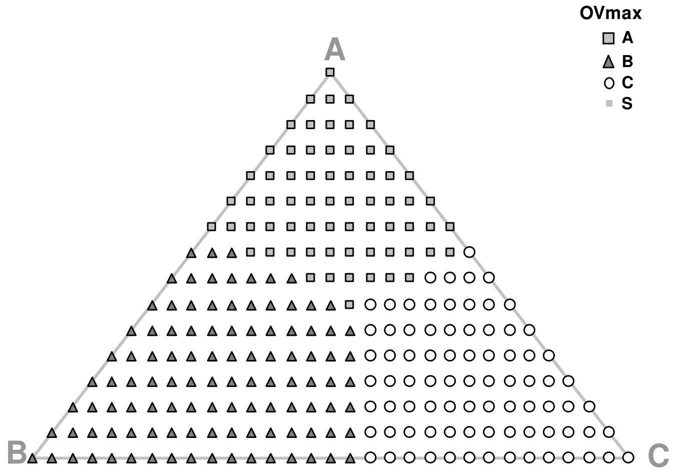
When comparing these two Figures, it can be seen that in the ideal case (Figure 6.8 -a) there are more points with higher OV_A, less points with higher OV_B and a region with $OV_{MAX} = OV_C$ at high concentrations of A and low concentration of B. This means that if we choose one of such compositions using the simplification of ideal liquid mixtures, we can have as a result one headspace with an odour value very different from what happens in the reality (non-ideal solutions).

In Figure 6.9 it can be seen the OV values for each component, OV_A, OV_B and OV_C, in the whole range of compositions represented in PTD, considering ideal and non-ideal solution. For comparison between them, all PTDs have the same scale: $0 \leq OV_i \leq 11000$. However, in the real liquid mixture assumption the ranges of each PTD are: $0 \leq OV_A \leq 9400$, $0 \leq OV_B \leq 6696$ and $0 \leq OV_C \leq 10990$. In the ideal liquid assumption the ranges are: $0 \leq OV_A \leq 4605$, $0 \leq OV_B \leq 6696$ and $0 \leq OV_C \leq 5265$. When the solution is ideal, Figure 6.9-a-b-c, the contour lines are parallel and perpendicular to the pure component because it is not taking into account the affinity to the other molecules in the mixture. Therefore, OV increase with the increase of the respective amount: OV_A is maximum at the corner A, where the amount of A is maximum ($x_A = 1$); OV_B is maximum at the corner B and OV_C is maximum at corner C.

This does not happen in non-ideal solutions and unexpected results appear, as can be seen in Figure 6.9-d-e-f. For example, the highest value of OV_A is not occurring at the highest amount of A, in the corner A, but in the region where there is a high concentration of C. This happens because the high polarity of C "pushes-out" the non-polar molecules of A. The same occurs with component C, with maximum OV_C occurring near corner A. Component B has a medium polarity, so the behaviour is more similar to the ideal one, being OV_B maximum at corner B.

(a)

IDEAL



(b)

NON-IDEAL

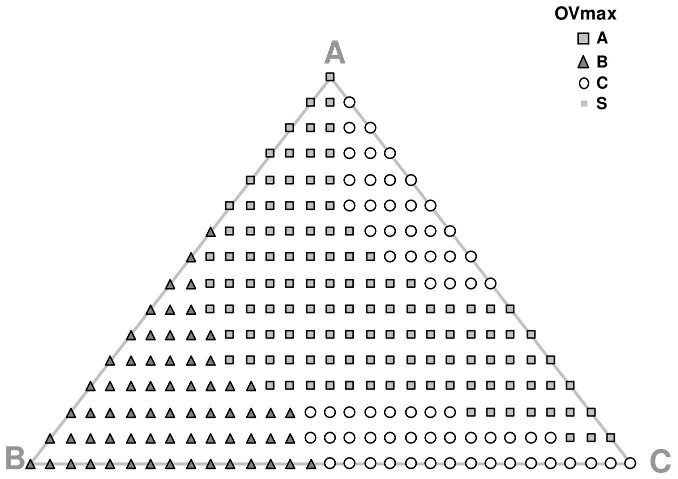


Figure 6.8 Perfumery Ternary Diagram for a perfume test mixture, composed of limonene (A), geraniol (B) and vanillin: (a) ideal solution (B) non-ideal solution.

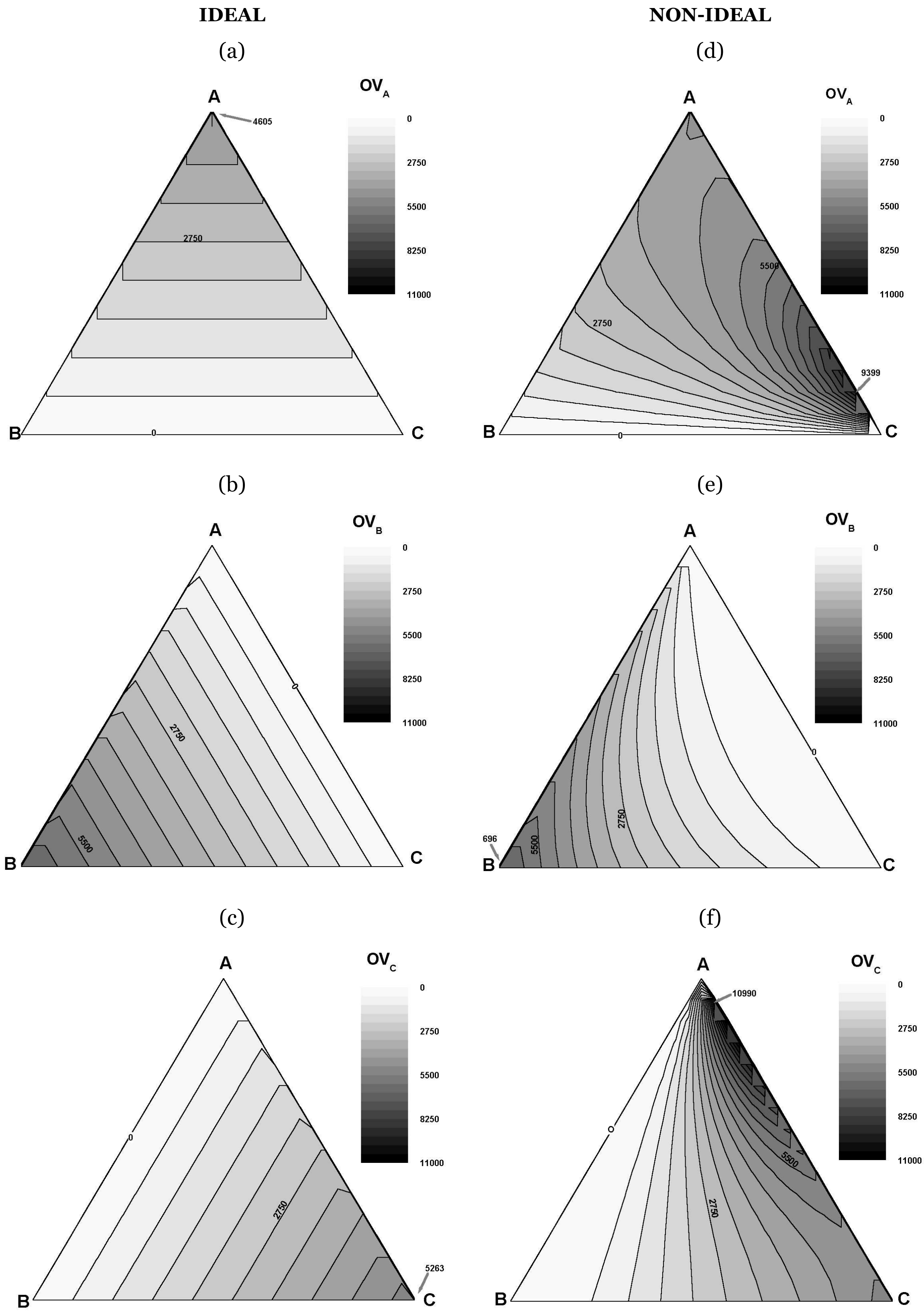


Figure 6.9 Variation of odour value (OV) as a function of the liquid composition and used to construct the perfumery ternary diagram of the test mixture with limonene (A), geraniol (B) and vanillin (C), for the ideal solution and non-ideal solution: (a) OV_A, ideal (b) OV_B, ideal; (c) OV_C, ideal; (d) OV_A, non-ideal; (e) OV_B, non-ideal; (f) OV_C, non-ideal.

6.3.4 Effect of ethanol on the PTD

Figure 6.10 shows the effect of the solvent ethanol, S, for the perfume test mixture composed of limonene, geraniol and vanillin, A+B+C, using *Perfumery Ternary Diagrams* (PTD) method, considering the non-ideal case. In each PTD shown, the molar fraction of ethanol is constant and is equal to: (a) $x_S = 0.2$, (b) $x_S = 0.5$, (c) $x_S = 0.7$ and (d) $x_S = 0.9$.

When comparing Figure 6.8 with Figure 6.10, it can be seen that the addition of a small amount of a polar solvent, such as ethanol, was enough to change the PTD profile. The points with $OV_{MAX} = OV_A$ (squares) increased since the medium became more polar, so with less affinity to this non-polar component and 'pushing' it out from the mixture. The upper region with $OV_{MAX} = OV_C$ disappeared because vanillin was more retained and OV_A increased. As the amount of solvent rises, illustrated by the sequence Figure 6.10 a-b-c-d, the points with $OV_{MAX} = OV_C$ (circles) and $OV_{MAX} = OV_B$ (triangles) gradually disappear, and then the points with $OV_{MAX} = OV_A$ decrease, while the area with $OV_{MAX} = OV_S$ is growing. This happens because the presence of ethanol in the solutions is retaining more the polar components and as it is also more volatile, the concentration of ethanol in the headspace increases and consequently its odour value also increases.

In perfumery, the effect of ethanol is important for two reasons: first, if we look to Figure 6.10 a-b-c-d, we are simulating the addition of ethanol in the final part of the perfume composition. For a given composition of A+B+C, we can determine the maximum amount of ethanol that can be added in order to have the OV_{MAX} corresponding to the desired component, but not the ethanol. This is important, for example, to make sure that when sniffing a perfume from a bottle, we smell the top note and never the ethanol. Secondly, if we look to these Figures in the reverse order Figure 6.10 d-c-b-a, it is simulated the fast evaporation of ethanol, generally some seconds after application. When no ethanol exists, the PTD corresponds to Figure 6.8-b.

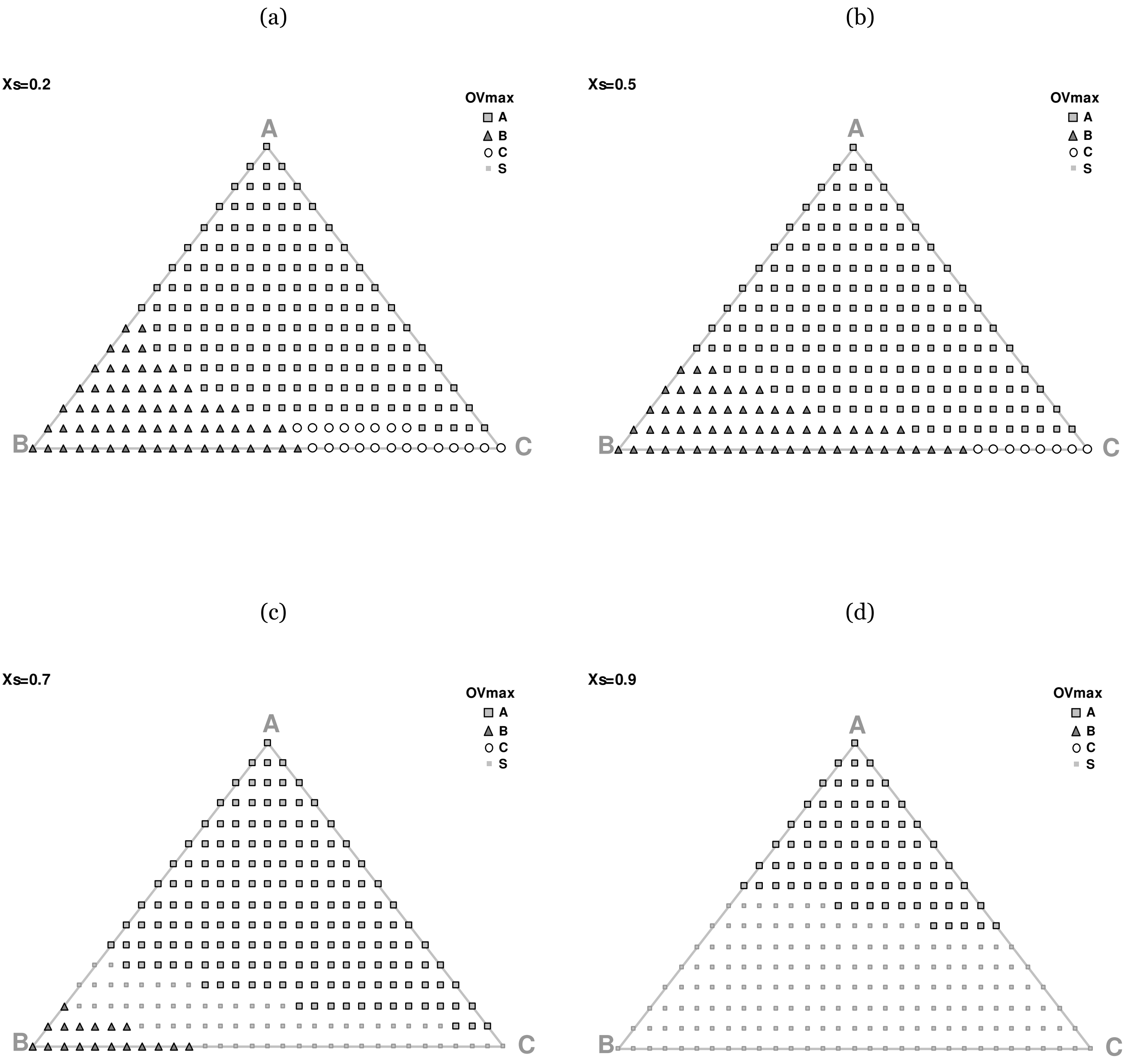

(a) (b) (c) (d)

Figure 6.10 Effect of ethanol on PTD of a perfume test mixture composed of limonene (A), geraniol (B,) vanillin (C) and ethanol (S): (a) Xs=0.2; (b) Xs=0.5; (c) Xs=0.7; (d) Xs=0.9.

6.4 New case studies

The developed Perfumery Ternary Diagram (PTD) theory was also tested using four additional systems:

System S1: benzaldehyde (A)+phenylethanol (B)+eugenol (C)+ethanol (S)

System S2: α-pinene (A)+linalool (B)+vanillin (C)+ethanol (S)

System S3: limonene (A)+geraniol (B)+tonalide (C)+ethanol (S)

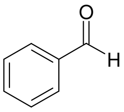
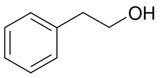
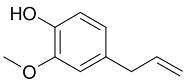
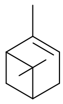
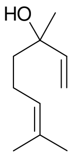
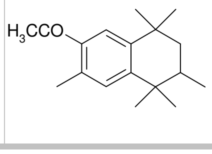
System S4: limonene (A)+linalool (B)+geraniol (C)+ethanol (S)

The physical constants at 25°C for these new aroma chemicals are shown in Table 6.2. The properties for limonene, geraniol, vanillin and ethanol were already presented in Table 6.1. The UNIFAC parameters are presented in Appendix A.

In Figure 6.11, Figure 6.12 and Figure 6.13 are shown PTDs for different amounts of solvent, for the ideal and non-ideal solution. Looking at Figure 6.11, representing System S1: A (benzaldehyde), B (phenylethanol), C (eugenol) and S (ethanol), it can be seen that benzaldehyde (A) is the dominant note for this system since there is a major area with points having $OV_{MAX} = OV_A$. This happens because benzaldehyde has a high value of saturated vapor pressure, P_i^{sat}.

Comparing the ideal and non-ideal solution, it can be seen that the PTD profiles are different. Considering the ideal solution, it is predicted that a certain composition will yield a $OV_{MAX} = OV_S$, for example, when actually it is smelling more strongly to A and not to solvent because $OV_{MAX} = OV_A$. This fact means that if we choose one of such compositions based on a PTD calculated using the simplification of ideal liquid mixtures, we could have as a result a perfume smelling stronger to ethanol and not the expected top, middle or base odour. The area of compositions where $OV_{MAX} = OV_S$ increases as the molar fraction of ethanol in the liquid also increases due to the increase of the odour value of ethanol.

Table 6.2 Physical constants and odour description of components used in the new four case studies, at 25 ^{o}C.

Component	**Molecular formula**	**M** (g/mol)	C_{thr_i} $^{(1)}$ (g/m^3)	P_i^{sat} $^{(1)}$ (Pa)	**Odour description**
benzaldehyde	C_7H_6O 	106.2	1.99×10^{-4}	1.06×10^{2}	bitter almonds
2- phenylethanol	$C_8H_{1o}O$ 	122.2	9.27×10^{-5}	4.8	rose
eugenol	$C_{10}H_{12}O_2$ 	164.2	8.06×10^{-5}	1.3	clove
α-pinene	$C_{10}H_{16}$ 	136.2	4.20×10^{-3}	3.9×10^{2}	pine
linalool	$C_{10}H_{18}O$ 	154.3	3.72×10^{-4}	3.1×10^{1}	flowery-fresh
tonalide	$C_{18}H_{26}O$ 	258.4	1.82×10^{-5}	6.7×10^{-5}	musk

(1) data from Calkin and Jellinek (1994)

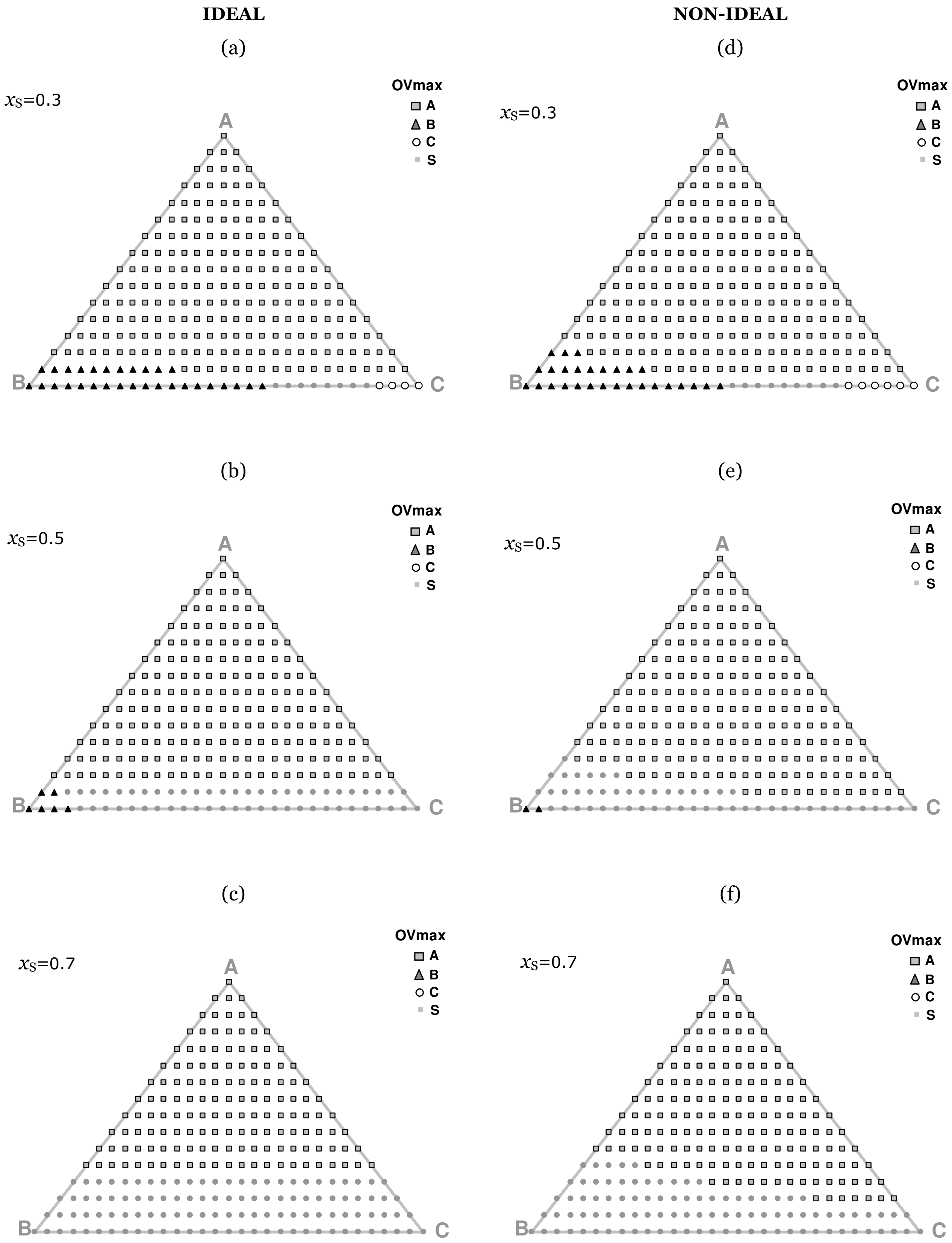


Figure 6.11 Effect of ethanol for System S1: benzaldehyde (A) ; 2-phenylethanol (B) ; eugenol (C); ethanol (S), considering the ideal and non-ideal solution, for different molar fractions of solvent (x_S).

Figure 6.12 represents the same study for System S2: A (α-pinene), B (linalool), C (vanillin) and S (ethanol). Looking for example to Figures 3c (left and right side) the reason for the difference in the number of points where $OV_{MAX} = OV_A$, where A is α-pinene, is due to its low polarity. If we consider non-ideal liquid mixtures, increasing the quantity of the highly polar solvent, ethanol, will "push-out" the non-polar component A more than would be expected when no molecular interaction is assumed in the ideal model.

Figure 6.13, represents the effect of ethanol on System S3: A (limonene), B (geraniol), C (tonalide) and S (ethanol). In this case there are no mixtures where $OV_{MAX} = OV_C$, because the saturated vapor pressure of C is much smaller than the other components. It can also be noticed that in this case, it is possible to choose a composition where $OV_{MAX} = OV_A$ even when the molar fraction of ethanol is high ($x_S = 0.7$). However, this behaviour is only possible to predict if we consider the non-ideal liquid mixture.

This information can be taken directly from the Perfumery Ternary Diagram (PTD) avoiding a long trial and error procedure to find the optimal mixture.

Considering now System S4 constituted by limonene (A), linalool (B), geraniol (C) and solvent ethanol (S), the effect of adding a base tonalide (D) to this system is shown Figure 6.14, using non-ideal solution. The molar fraction of ethanol used was $x_S = 0.5$. Comparing Figure 6.14 a and b, it can be seen that the number of points where the maximum odor value is that of ethanol increased. This happens because tonalide is a very non-polar component and will "push-out" the more polar components in the mixture, in this case, the ethanol. On the other hand, when tonalide is increased, although again the compositions where the OV have a maximum start in the middle of the Perfumery

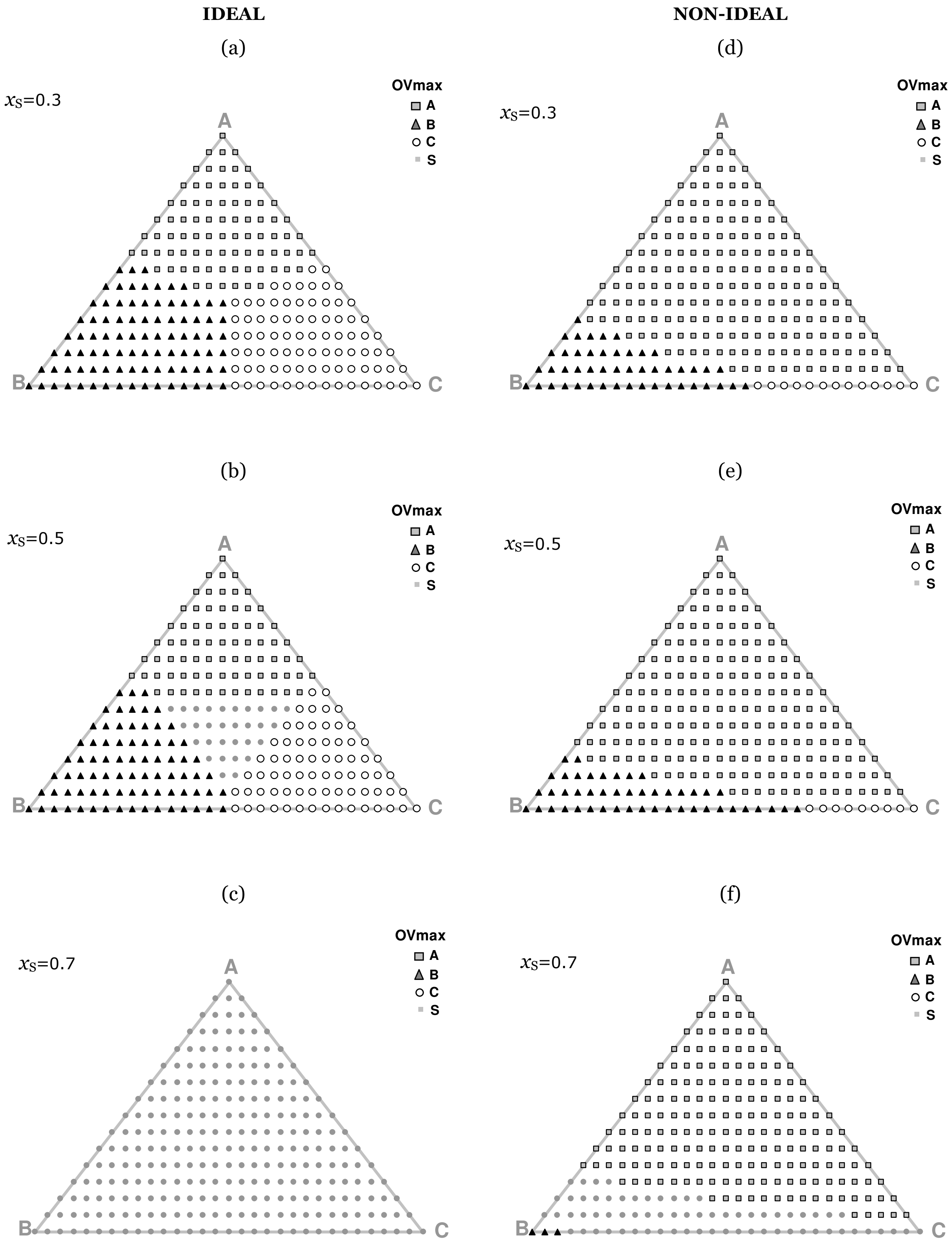


Figure 6.12 Effect of ethanol for System S2: α-pinene (A) ; linalool (B) ; vanillin (C); ethanol (S), considering the ideal and non-ideal solution, for different molar fractions of solvent (x_S).

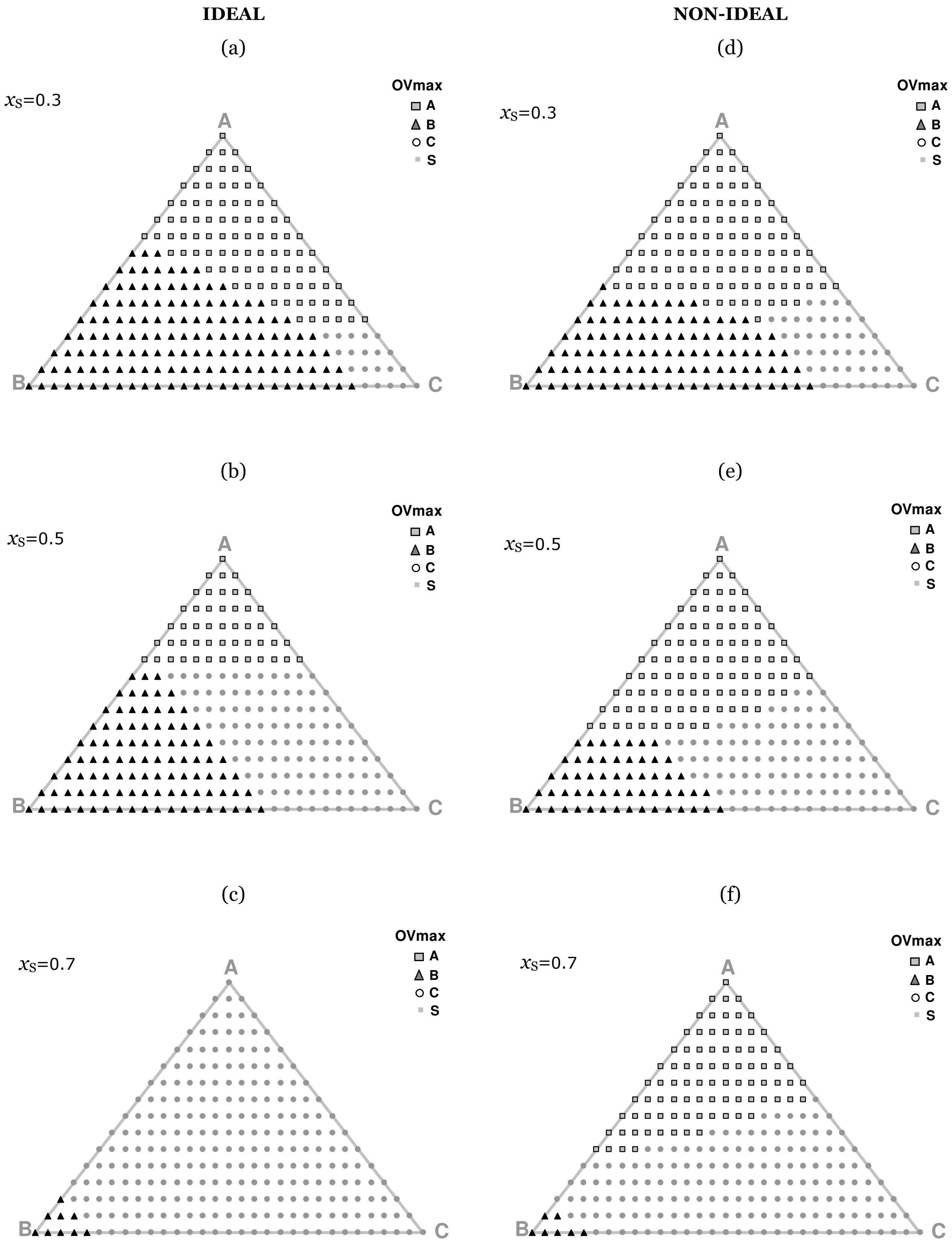


Figure 6.13 Effect of ethanol for System S3: limonene (A) ; geraniol (B) ; tonalide (C); ethanol (S), considering the ideal and non-ideal solution, for different molar fractions of solvent (xs).

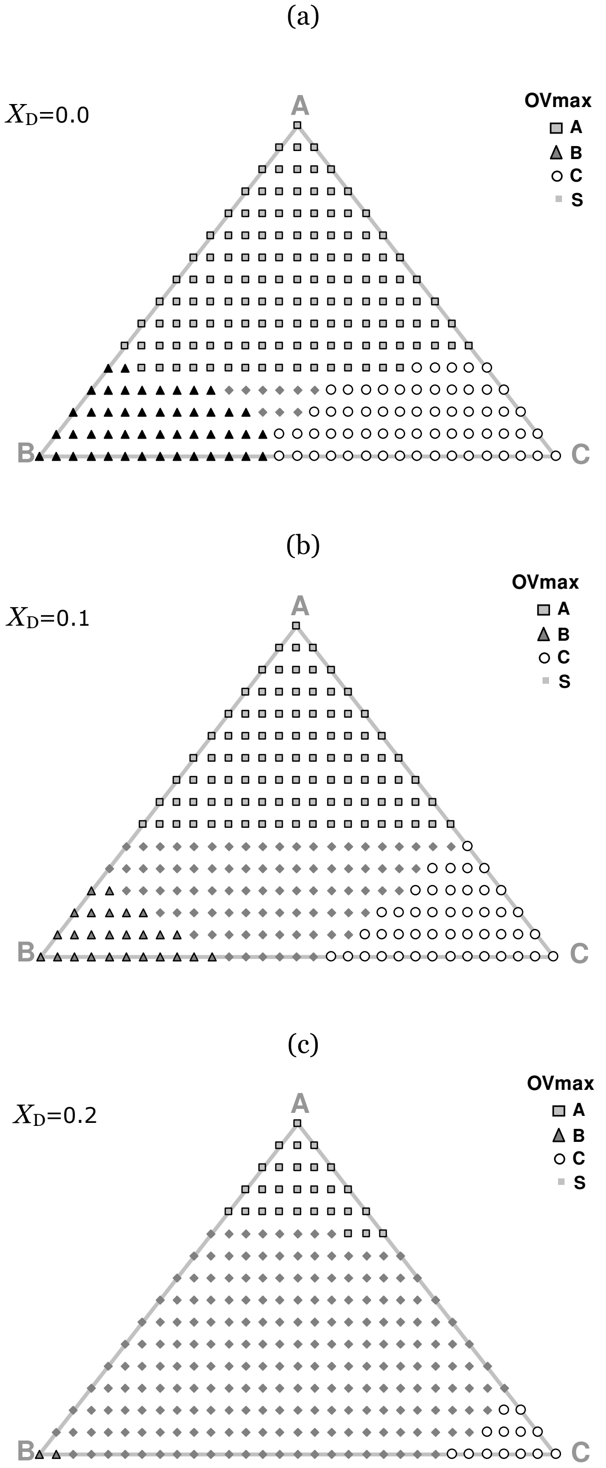


Figure 6.14 Effect of adding a base note tonalide (D) to System S4: limonene e (A) ; linalool (B) ; geraniol (C); ethanol (S), considering the non-ideal solution, for different molar fractions of tonalide (x_D) and with a constant molar fraction of ethanol x_S=0.5.

Ternary Diagram (PTD), when the percent of tonalide increases, the points where the odour value maximum is that of ethanol expands is the direction of the three corners of the PTD. This happens for two reasons. First, because tonalide is a non-polar component, such as limonene. This means that tonalide tend to retain limonene in solution more strongly than it happen when adding ethanol. Second, because the difference in polarities between ethanol and tonalide increases as more tonalide is added, and then the tendency for ethanol leave the solution increases as the liquid solution becomes less polar.

6.5 Design of a perfume: how to choose the initial mixture?

As already mentioned, a typical perfume is composed of ethanol and a mixture of three types of fragrant components: top, middle and base notes. When a perfume is applied, it is supposed that the different notes can be noticed "separately" after some time.

It was also considered above that a component i is perceived strongly by human nose when its odour value, OV_i in the headspace above the liquid volatile mixture is higher than those of the other components.

The composition that the perfumer can choose using the concept of Perfumery Ternary Diagram depends mainly on what kind of perfume he intends to produce. Traditionally, there are two rules that are met in order to optimize the perfumes behavior and for which the PTD can give an important contribution:

(i) top note should be smelled strongly in the bottle or shortly after application. This rule can be translated as: $OV_{\max} = OV_A$;

(ii) base note should be noticed long after application. It is used as a fixative of the top and middle notes and can last many hours (sometimes, days or months). Can be chosen in order to determine the persistence of the perfume.

(iii) ethanol should not be noticed: $OV_S < OV_{\max}$.

This information can be taken directly from the Perfumery Ternary Diagram (PTD) avoiding a long trial and error procedure to find the optimal initial mixture.

Other important information can be taken from the Perfumery Ternary Diagram (PTD). If one wants to have a less intense top note, a composition far from the corner A should be chosen. On the other hand, to have a high intensity top note (Fresh or Citrus perfumes, for example) a composition closer to the corner A is used. Also PTD can be used to find the maximum fraction of ethanol to be used, for a given composition of concentrated perfume.

More than ever perfumers try combinations with the more innovative characteristics as possible in order to offer new scents to their clients and be ahead from their competitors. Many other situations can be easily predicted using the developed *Perfumery Ternary Diagram* theory, as many as the combinations to make one perfume.

6.6 Conclusions

A methodology to predict the behavior of binary and ternary perfume mixtures in a solvent base was developed. This technique is based on the concept of Perfumery Ternary Diagrams, which allows a fast theoretical prediction of the headspace odour value, OV, for any combination of these materials in the corresponding liquid solution, without making too many experimental trial and error combinations. PTD can be used as compounding tool for making the perfume mixtures. The concept of PTD was applied to the perfume test mixture limonene (top note), geraniol (middle note) and vanillin (base note), in an alcoholic solution, using ethanol as solvent. The application of PTD model was also extended to four systems composed of other aroma chemicals.

As we can see in the test example shown in this work it is not straightforward to look at the values of vapor pressure and threshold of one odorant component and define to which note – top, middle or base - it belongs to because it depends on the other molecules in the mixture. These observations mean that the choice of the right initial liquid mixture is very complex, because there exists always a simultaneous effect of molecular polarity and component's concentration. However, the described methodology of perfumery ternary diagram, PTD, shows a simple way of studying the effect of odorant chemicals when they are present simultaneously in a mixture.

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CHAPTER 7

EXPERIMENTAL VALIDATION OF PERFUMERY TERNARY DIAGRAM METHODOLOGY

In the previous chapter a new methodology based on *Perfumery Ternary Diagram* (PTD) to predict the odour value of a perfume mixture was introduced. The PTD method is based on odour values, which in turn are calculated from measurable data, namely headspace or gas concentrations and odour threshold values. In this chapter, these data were measured experimentally, using an olfactometer and headspace analysis, in order to determine OV values and compare with OV values predicted by PTD model.

7.1 Introduction

The perfumery materials have a wide range of odour threshold values, which vary several orders of magnitude between them, as for example, 440 ppb for limonene and 0.03 ppb for vanillin (Calkin and Jellinek, 1994), showing that the nose responds quite differently to the fragrances. To date, it is not possible to predict the odour of a molecule, though there are some theories that try to explain how the sense of smell works (as already mentioned in Chapter 2).

As the determination of odour threshold values depend on a sensory response and on the experimental conditions, the values found in the literature vary greatly, existing differences of several orders of magnitude. For example, for geraniol there are values between 1.20×10^{-5} mg/m^3 and 1.29 mg/m^3 (Van Gemert, 1999). Another example is shown in Table 7.1 for ethanol, the most common media for perfumes, where there are several values of odour threshold determined that vary from 10^{-1} to 10^{+3}, a variation that covers 4 orders of magnitude. Therefore, it is necessary that each perfumer determines his own odour threshold values and build his own database based on his sensory response at perfectly

Table 7.1 Odour threshold values found in the literature for ethanol (values from compilation by Van Gemert, 1999).

Reference	**Odour threshold (mg/m^3)**
Dravnieks &Laffort (1972)	6.40E+02
Dravnieks (1974) [d]	1.35E+03
Nishida et al.(1979) [d]	3.02E+02
Anon. (1980) [d]	6.40E-01
Anon. (1980) [r]	1.16E+01
Naus (1982) [d]	2.00E+00
Naus (1982) [r]	2.00E+01
Cristoph (1983) [r]	8.95E+00
Cometto-Muñiz & Cain (1990)	1.54E+02
Scharfenberger (1990)	9.88E+02

d - detectable; r - recognized

controlled conditions, since his work will depend on his sensitivity. It is like making the “calibration curve” of his nose. Taking this into account, in this work, there were measured our own odour threshold values, using an olfactometer, at our working conditions.

As this work is about perfumes, knowing the smell of perfumery materials and training the olfaction to recognize the fragrances was a very important part of this work. An extensive database of perfumery materials, including essential oils and aroma chemicals, was determined experimentally.

7.1.1 Odour threshold concentration

The sense of smell is accomplished through olfactory and trigeminal nerves that enable perception and sensation reactions, respectively. During normal breathing 10% of inhaled air passes by olfactory receptors, which increases to more than 20% in a sniffing action. The solubility in water of the odorants and the mass concentration of molecules are determinant factors for olfactory response: water solubility is related to mass transfer to the mucus layer and then to the olfactory receptors, while the response magnitude depends on concentration. The design of the equipment for the olfactory measurements must consider these factors so that the sensory response is meaningful. Moreover, as the sensory response vary from person to person (women and younger people are more sensitive) and depends on the experimental conditions at which they are measured (experimental methodology, purity of fragrant materials, fragrance base in which is evaluated) (Leffingwell&Associates, 2004), it is necessary to get a sizeable amount of data in order to get an average that is representative. The methods are described in several international standards (ASTM-E679-91, 1991; AWMA-EE-6, 2002; EN13725, 2003; VDI3881, 1980).

Olfactometry is a sensory method based on the olfactory evaluations that is defined according to the German Standard VDI 3881 (1980) as “the controlled presentation of odorants and the registration of the resulting sensations in man”.

The olfactometer is the equipment used to perform the olfactory measurements by making precise dilutions of the odorant sample with neutral air in a defined ratio, in a sequential way and at a fixed dilution step. There is a group of panellists that will smell and detect the presence of the odorant sample, using the human nose as a detector to determine (VDI3881, 1980):

- odour threshold concentrations of individual materials and mixtures in a laboratory, using samples of know concentrations and determining the dilution factor;
- unknown concentrations of odorous materials in ambient air or at emission sources, knowing their threshold values and dilution factor of the smelled sample.

Odour is measured both qualitatively and quantitatively, having four main dimensions (EN13725, 2003):

- detectability or threshold, already described;
- intensity: perceived strength of odour sensation, determined by comparison with a standard, closely related to the concentration of the odour molecule;
- quality or character: classification of odours according to a list of descriptors (green, floral, woody, fruity, etc);
- hedonic tone: evaluation of degree of pleasantness or annoyance of an odour, according to a scale of -10 (most unpleasant), 0 (neutral) and +10 (most pleasant).

Main qualitative measurements consist in quality, intensity and hedonic tone, whereas quantitative measurements are odour threshold values. In this work, only the quantitative measurement of odour thresholds will be considered in order to quantify the odour performance of fragrances based on the concept of odour value, defined in Chapter 6.

The measurement of odour threshold can be made considering the concentration of the fragrant component in a solution, in the headspace or directly in the inhaled air (Calkin and Jellinek, 1994). Odour threshold values in air are the most interesting since they are independent of the solvent used. On the other hand, the relative threshold values obtained for different solvents indicate its performance in different application environments.

Odour threshold concentration is determined according to the standards VDI 3881, EN13725 or ASTM E679-91. In practice, the measurement consists of a dilution series of a gas sample of known concentration, where the threshold odour concentration is calculated by determining the dilution factor at which:

- the odour starts to be sensed if the concentration is presented in ascending order (decreasing dilution factor), or

- the odour stops to be detected if the concentration is presented in descending order (increasing dilution factor).

Figure 7.1 shows a scheme of the measurement procedure for the determination of odour threshold values.

The sample is usually presented in ascending concentration series in order to avoid adaptation phenomena of the sense of smell. There are used the following methods:

- triangular forced-choice: 3 samples are presented to the panellists, one is the odour sample and the other two are neutral air, and they have to distinguish the one that is different (corresponding to the sample);
- method of limits: consist of a yes/no questioning, in which the panellist is asked if he/she can smell or not the odour sample.

The odour threshold concentration is determined as the concentration that leads to an odour impression in 50% of the panellists. There are defined two other statistical parameters: reaction limit (odour impression in 16% of the panellists) and determination limit (odour impression in 84% of the panellists). The ratio of these two parameters is indicative of the dispersion of the results obtained.

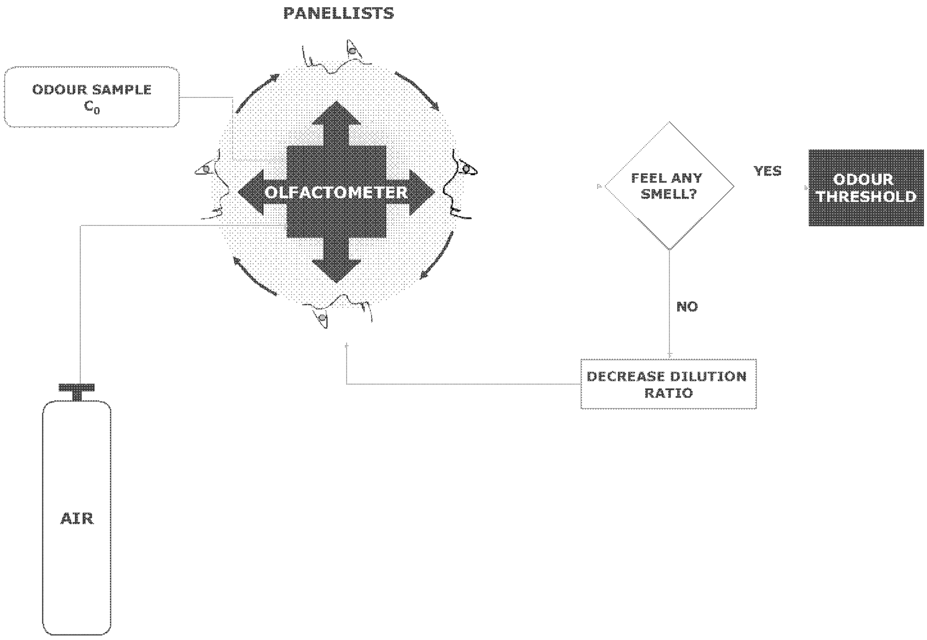


Figure 7.1 Scheme of the experimental measurement of odour threshold values.

7.1.2 Headspace concentration

Any fragrant component to be perceived must be in the gas phase so that it can reach the olfaction sense and be recognized as a smell. However the components in a perfume mixture have different volatilities and therefore the composition in the gas phase is significantly different from the liquid composition. Moreover, there are interactions between the components, which increase or decrease the volatility of certain components.

There are two main techniques using gas chromatography to quantify the vapour phase in equilibrium with a liquid phase (static headspace) (Zehentbauer *et al.*, 2004): direct injection using a gastight syringe (S-HS) or adsorptive fiber as solid phase microextraction (SPME).

Direct injection is the simplest technique and the one that measures the “true” headspace concentration since there is no distortion introduced by concentration techniques (McNally and Grob, 1985; Weston *et al.*, 1992). However, when dealing with low volatile components, with very small headspace concentrations, there is not enough sensitivity to detect them by this method.

On the other hand, SPME has the important advantage of combining the isolation and the enrichment (concentration of amount) of the components of interest in a single step in the sample preparation for analysis (Yang and Peppard, 1994; Zhang *et al.*, 1994). The quantification is more complicated since the results are significantly influenced by the nature of the component (structure, polarity), the overall headspace composition and experimental sampling conditions, such as time of extraction, temperature, fiber material, desorption conditions at GC injector and distance from liquid-gas interface.

7.2 Experimental

7.2.1 Measurement of odour threshold values

In this work, the detection odour thresholds were determined by using a specific equipment for olfactory measurements – the olfactometer. The experimental procedure and conditions are described next.

Materials

The following pure fragrances or aroma chemicals were used: limonene (Fluka, 98%), α-pinene (Fluka, 99%), benzaldehyde (Merck, 99%), linalool (Fluka, 97%), linalyl acetate (Fluka, 95%), benzyl acetate (Aldrich, 99%), citral (Aldrich, 95%), citronellol (Fluka,90-95%), geraniol (Sigma, 98%), 2-phenylethanol (ACROS, 99%), phenylacetaldehyde (ACROS, 80-90%), α-hexyl cinnamaldehyde (Aldrich, 85%), eugenol (Aldrich, 99%), musk galaxolide in 50% DEP (Aldrich), vanillin (Fluka, 98%), ethanol (Merck, 99.9%), Iso E Super (IFF, USA), lilial or lilestralis (AFC), hedione (Firmenich, Ch), methyl ionone (Quest, NL), lyral (IFF, USA), rubarbus (PFW, NL). Three essential oils were also used: rose geranium oil (hydrodistilled oil, obtained in this work), vetiver oil (MON, ES) and rose oil (Roig Pharma, BE).

Equipment

The equipment used to perform the olfactory measurements was an Olfactometer Ecoma model T07, shown in Figure 7.2.

The principle of functioning of the olfactometer is accordingly with the standards VDI 3881 and EN 13725. The odour sample is prepared in a sample bag (see Figure 7.3), which is then connected to the olfactometer.

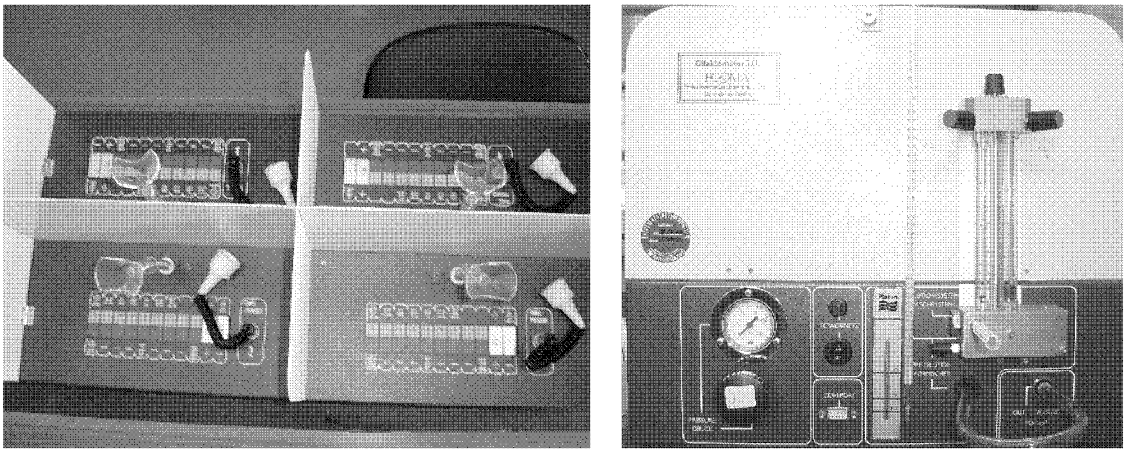


Figure 7.2 Olfactometer equipment.

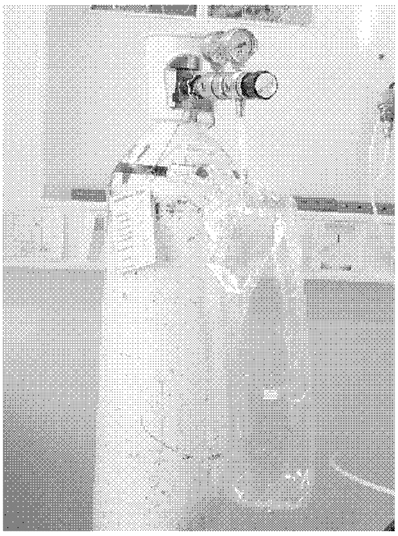


Figure 7.3 Preparation of odour sample bag.

When the measurement starts, the odour gas is sucked from the sample bag through the pre-mixing chamber and the flow is controlled by the flowmeters and needle valves. In the gas jet pump, the sample is mixed intensely with neutral air in the proportion set by the needle valves. The dilution ratio is set at the highest dilution factor in a range from 640 to 2.5 without pre-dilution. For higher dilution factors, a pre-dilution has to be set as 1:25, 1:50 or 1:100, increasing the range up to 64 000.

The diluted odour flow is directed alternatively to the sniffing ports, from Panellist 1 to Panellist 4, by means of a rotative valve. While one of the panellists is smelling the odour gas, the panellist in the opposite position is smelling neutral air and the other two are in the expiration phase. The breathing frequency is set by on/off led signals (on – to inspire, off – to expire). The panellist should press the button when detects an odour. There are 20% of random blanks that correspond to neutral air instead of odour sample, to check if there is any tendency for guessing.

The measurement is run automatically and controlled by the software. There is a test leader who sets the valves manually to the dilution indicated by the software and gives indications to the panellists. If no odour is detected, the next dilution step is set. The measurement sequence stops after two consecutive positive answers by at least 50% of the panellists, or when it reaches the end of the sequence at the lowest dilution factor (highest concentration). Each measuring test comprises three measuring sequences, with a rest period of 60 s between them. In the end, the results are saved in a file.

The system requires a supply of oil-free compressed air, at a maximum inlet pressure of 4 bar. The operating pressure regulated by the manometer of the olfactometer is 1.5 to 1.9 bar. The necessary air flow is 50 l/min.

The olfactometer is used in the conditions shown in Table 7.2.

Preparation of fragrant samples

The sample bag was closed, labelled and partially filled with neutral air just to give it a cylindrical form. To prepare the fragrant gas sample, a known volume of pure aroma chemical was inserted in the bag through the tube, using a GC or HPLC syringe, as shown in Figure 7.4. The sample bag was filled completely with neutral air and immediately closed. The fragrant gas solution was left to equilibrate for at least one hour, at room temperature of 25 °C.

The volume of the sample bag was determined by filling the sample bag with water and measuring the volume of liquid contained in the bag. It was determined that the volume of the sample bag was 8.6 l.

Table 7.2 Conditions used for the olfactory measurements

Parameter	Value set
dilution step*	2
dilution range	2.5 - 64000
measuring sequences	3
interval between sequences	60 s
number of panellists	1 - 4
breathing time	2.2 s
air flow rate (inhaling)	1.2 m^3/h (1.5 bar)
operating air	medicinal air
inlet gas pressure	3 bar
operating pressure	1.5 bar
room temperature	25 °C

* dilution step=2 means that the actual dilution ratio is set to half of the previous one, in a measuring sequence

Figure 7.4 Preparation of fragrant gas solution in the sample bag.

The concentration of the fragrant component in the sample bag was calculated based on the added volume of the aroma chemical:

$$C_0 = \frac{V_0 \cdot \rho}{V_{bag}} \times 10^6 \qquad (7.1)$$

where C_0 is the gas concentration of the aroma chemical in the sample bag (mg/m^3), V_0 is the volume of aroma chemical added to the sample bag (m^3), ρ is the density of the aroma chemical (kg/m^3) and V_{bag} is the volume of the sample bag (8.6×10^{-3} m^3).

Measurement of odour threshold values

Three sets of data were obtained: two sets of odour threshold values obtained by two panellists, individually, and one set with a panel constituted by 4 people (co-workers in the laboratory), as can be seen in Figure 7.5. The measurements were carried out at an ambient controlled temperature of 25 °C, at the same time of the day and the procedure of preparing the samples was the same for all the experiments. The 4 panel members were constituted by three women and one man, with ages between 26 and 31 years old.

Calculation of odour threshold values

The dilution ratio at threshold, Z_{thr}, is calculated after obtaining 2 consecutive positive answers as follows:

$$Z_{thr} = \sqrt{Z_N \times Z_{N-1}} \qquad (7.2)$$

Figure 7.5 Measurement of odour threshold value using a panel of 4 people.

where Z_N is the dilution ratio used at the first positive answer at step dilution N and Z_{N-1} is the dilution ratio of the previous dilution step at threshold, *N-1*. This result corresponds to the geometric mean of the last step when no odour is smelled (Z_{N-1}) and the first step when the odour is detected (Z_N). A more detailed description of the calculation performed is presented in Appendix C.

The odour threshold value (mg/m3), C_{thr}, is finally calculated by:

$$C_{thr} = \frac{C_0}{Z_{thr}} \tag{7.3}$$

7.2.2 Headspace analysis

The headspace analyses were used to determine the concentration in the gas phase above the perfume test mixture and to determine the vapour pressure of the pure components (limonene, geraniol, vanillin, ethanol) at 25 °C.

Materials

A solution of a mixture of limonene (Fluka, 98%), geraniol (Sigma, 98%) and vanillin (Fluka, 98%) in ethanol (Merck, 99.9%) was prepared, as a test perfume mixture with a top citric note, middle rose floral note and base sweet vanillin note, respectively. The molar fraction of ethanol was 0.52 and the other components were in equimolar quantities of 0.16, which corresponded to a concentration in mass of approximately 25% in each component. A 4 ml aliquot of the perfume mixture was placed in a 20 ml glass vial capped with a Teflon valve (Supelco) or with aluminium crimp caps with Pharma-Fix septum. The samples were equilibrated overnight before the first headspace analysis and 1 hour between analyses, at room temperature of 25 °C. Each sample was analysed in duplicate.

For the determination of vapour pressure values of the pure components, an amount of 4 ml of each pure component was placed in a 20 ml headspace vial. The samples were left to equilibrate overnight, at room temperature of 25 °C.

Calibration curves

For the quantification of the volatile components (limonene, geraniol, vanillin and ethanol), a calibration curve for each component was determined by the external liquid calibration method: finding the linear correlation between GC peak areas and known quantities of injected mass.

Standard solutions of limonene, geraniol and vanillin in ethanol were prepared with concentrations of 0.01 g/l, 0.05 g/l, 0.1 g/l, 0.5 g/l and 1 g/l. Injections of 0.1 μl were used to prevent vapour overpressure and subsequent loss of sample (Tudori *et al.*, 2000). Each calibration point corresponded to the average of three injections. The calibration curves are linear in the range of concentrations considered, having correlation coefficients of 0.999 for all the components (see Figure 7.6).

Direct headspace analysis by gastight syringe

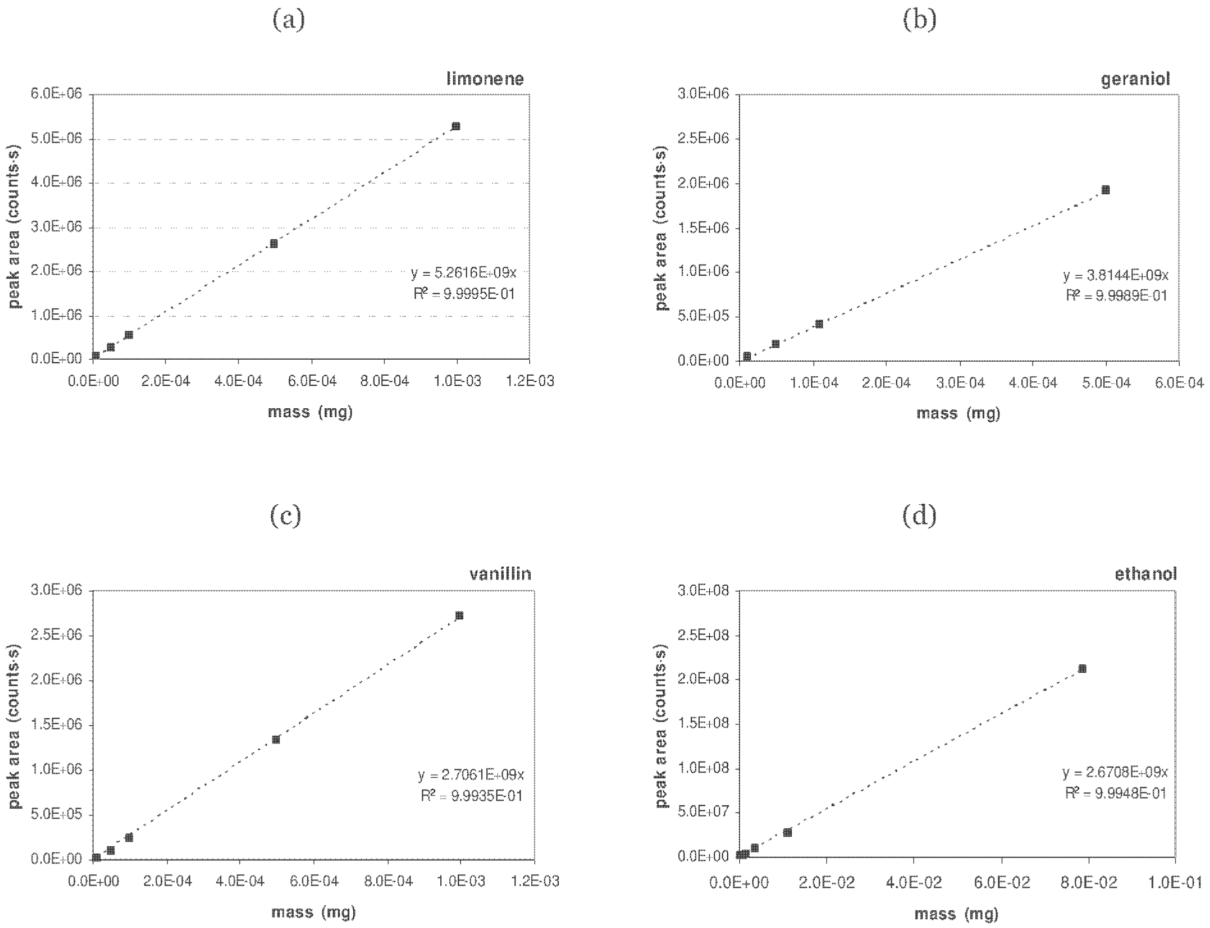
A gastight syringe (SGE, 1.0 ml) was used to take 500 μl of headspace sample and inject it into the Varian GC. The syringe was flushed several times with clean air before and after the sampling, to prevent carry over of the sample. The sample was withdrawn and injected at a rate of 100 μl/s.

SPME analysis

SPME headspace sampling was done by a manual holder (Supelco), with a 50/30 μm divinylbenzene-carboxen-polydimethylsiloxane (DVB/CAR/PDMS) Stableflex, 2 cm long fiber (Supelco). This is a porous bipolar adsorption-type fiber that extracts both polar and nonpolar components and is suitable for the analysis of an expanded range of components (C3-C20, MW 40-275), including flavours and odour components, according to supplier's information.

The fiber was conditioned at least 20 min at 250 °C before analysis. Three sampling times were used: 30 s, 1 min and 1 hour. After sampling time, the fiber was withdrawn and inserted immediately into GC injection port for desorption (5min, 250 °C, splitless). The fiber was kept in the injector during analysis for cleaning and to be ready for use for next sampling.

The extraction time profiles for the 4 components were obtained by exposing the SMPE fiber to the headspace of pure components and solutions of pure components in ethanol (volume of headspace equal to 16ml), for 15 s, 30 s, 1 min, 5 min, 20 min and 60 min.


(a)

(b)

(c)

(d)

Figure 7.6 Calibration curves for quantification by GC analysis for the components used in the perfume test mixture: (a) limonene; (b) geraniol; (c) vanillin; (d) ethanol.

GC analysis

Gas chromatography GC/FID was carried out using a Varian CP-3800 instrument equipped with a split/splitless injector and a capillary polar column from Chrompack (CP-Wax 52 CB bonded fused silica, 50 m × 0.25 mm i.d. , 0.2 μm film thickness). The oven temperature was programmed isothermal at 50 °C for 5 min, to 70 °C at a heating rate of 2 °C/min, to 200 °C at 10 °C/min and then held isothermal for 20 min. The injector was set at 250 °C, splitless for the initial 5 min and afterwards with a split ratio of 1/50. The FID detector temperature was 250 °C. The carrier gas was helium He N60, at a constant flow rate of 1 ml/min.

Calculation of headspace concentrations

The headspace concentration was calculated by:

$$C_{hs} = \frac{m}{V_{inj}} \tag{7.4}$$

where C_{hs} is the headspace concentration (g/m^3), m is the amount in the headspace (mg) and V_{inj} is the injected volume (m^3). The amount m was determined through the GC peak and the respective calibration curve.

In the gas-tight analysis the injected volume was 0.5 ml. For SPME analysis, the headspace concentration was determined as the ratio of the mass desorbed from the fiber (determined also by GC peak and the respective calibration curve) and the headspace volume (16 ml, total gas volume in the vial).

Determination of vapour pressure values

A volume of 600 μl of the headspace above the liquid sample of pure component was injected into GC by a gastight syringe (SGE, 1.0 ml), at a withdrawal and injection rate of 100 μl/s. Each sample was analysed in triplicate. The vapour pressure P_v was then calculated by the following equation, based on the ideal gas law:

$$P_v = \frac{C_{hs}}{M} RT \tag{7.5}$$

where C_{hs} is the headspace concentration (g/m^3), M is the molar mass (g/mol), R is the gas constant (8.314 J mol^{-1} K^{-1}) and T is the temperature (298 K).

7.3 Results

7.3.1 Determination of odour threshold values

The odour threshold values determined experimentally at 25 °C are shown in Figure 7.7. The values correspond to the odour thresholds detected by each panellist, for each measurement. The components with more points in the graphic were determined in duplicate. The data obtained for each component show a great variability, although they were obtained by the same panel, at the same conditions and keeping the same procedure. These results confirm the difficulty in obtaining consistent sensory data since the olfactory sensitivity changes considerable from person to person. Consequently, there will be some people that will smell ethanol or smell more strongly some component, for example in the perfume, if they have a low threshold and others will not even feel it because their threshold is higher.

In the same plot, the experimental results are compared in the values found in the literature (Van Gemert, 1999). It can be seen that the determined odour thresholds fall in the range of the literature values, which in turn also vary over a wide range of values.

Therefore, the work of a perfumer should be based on his own odour threshold, since this is the only meaningful reference for him to evaluate the smell of his perfume compositions. In this work, the odour thresholds used were the ones determined by panellist P1 (see Table 7.3).

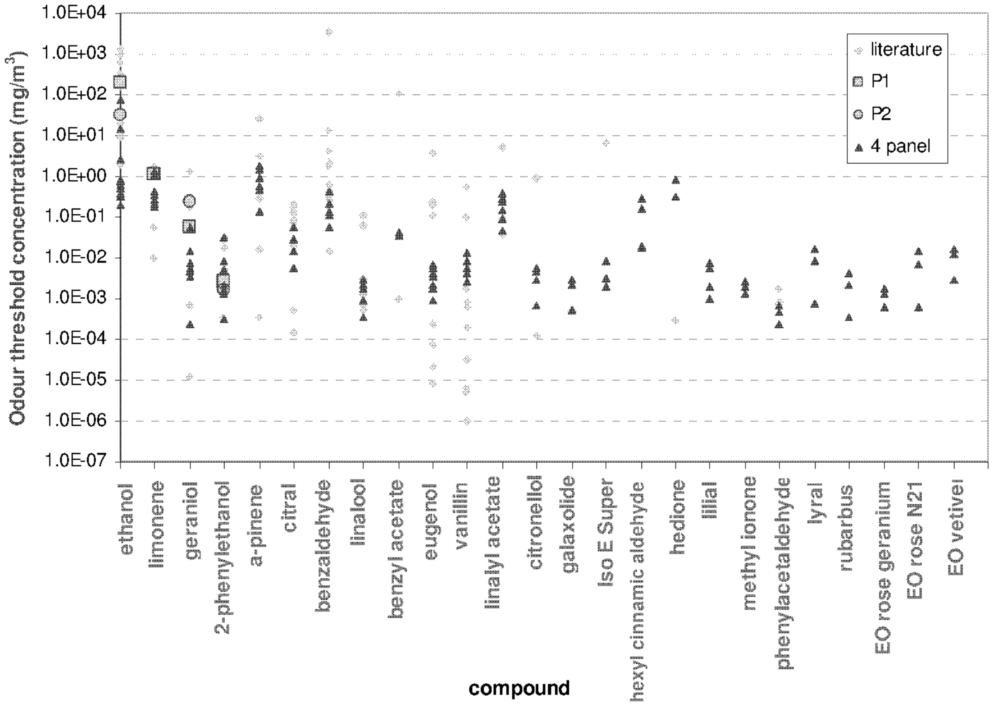


Figure 7.7 Odour threshold values determined experimentally and comparison with values found in the literature (Van Gemert, 1999).

Table 7.3 Data for the components used in the perfume test mixture.

	Component	C_{thr_i} (1) (g/m^3)	P_i^{sat} (2) (Pa)
A	limonene	1.09×10^{-3}	5.68×10^{1}
B	geraniol	5.75×10^{-5}	3.53×10^{-1}
C	vanillin	5.15×10^{-6}	2.70×10^{-2}
S	ethanol	2.04×10^{-1}	3.76×10^{3}

(1) data obtained experimentally by panellist P1

(2) data obtained by direct headspace analysis

7.3.2 Comparison of headspace analysis methods

The odour performance of a perfume test mixture composed of limonene, geraniol, vanillin and ethanol (A+B+C+S) was characterized using odour values that are based on measurable parameters of gas-phase concentration and odour threshold values. The headspace concentrations were determined using two techniques coupled with GC/FID: direct headspace injection by gastight syringe and SPME. The vapour pressures that were determined experimentally are shown in Table 7.3 .

The composition profiles of the perfume test mixture at both liquid and gas phase and using different headspace techniques are shown in Figure 7.8. In order to allow the comparison between the concentrations of the components, which vary in a range of several orders of magnitude, the charts are represented in logarithmic scales.

All the components had approximately the same concentration in the liquid phase. However, due to their different volatilities, they evaporated at different rates and therefore the headspace concentration was quite different.

The “true” headspace concentration was given by the direct headspace injection. All the components were detected by GC, including the low volatile vanillin. As expected, the most volatile components limonene and the solvent ethanol were the ones with the highest amount in the gas phase, having a concentration of 3 to 4 orders of magnitude higher than the other two.

Considering the odour performance of the perfume test mixture, the higher concentration of ethanol in the headspace might lead to the conclusion that the component that would be smelled with more intensity would be that of ethanol. However, as can be seen in Figure 7.8-d, limonene showed the maximum odour value, followed very closely by the OV of vanillin, because although its headspace concentration is low, its odour threshold is also very low (see Table 7.3). This means that limonene was the component perceived with highest intensity, but vanillin was also felt relatively strong. In fact, the perfume test mixture smelled to lemon candies, with very sweet (vanilla) lemon odour, and the ethanol was not detected.

Figure 7.9 shows the SPME extraction time profiles for the components present in the perfume test mixture, for the headspace above pure components and above a binary mixture of the component and ethanol. The concentrations obtained by SPME were normalized by the concentration determined by

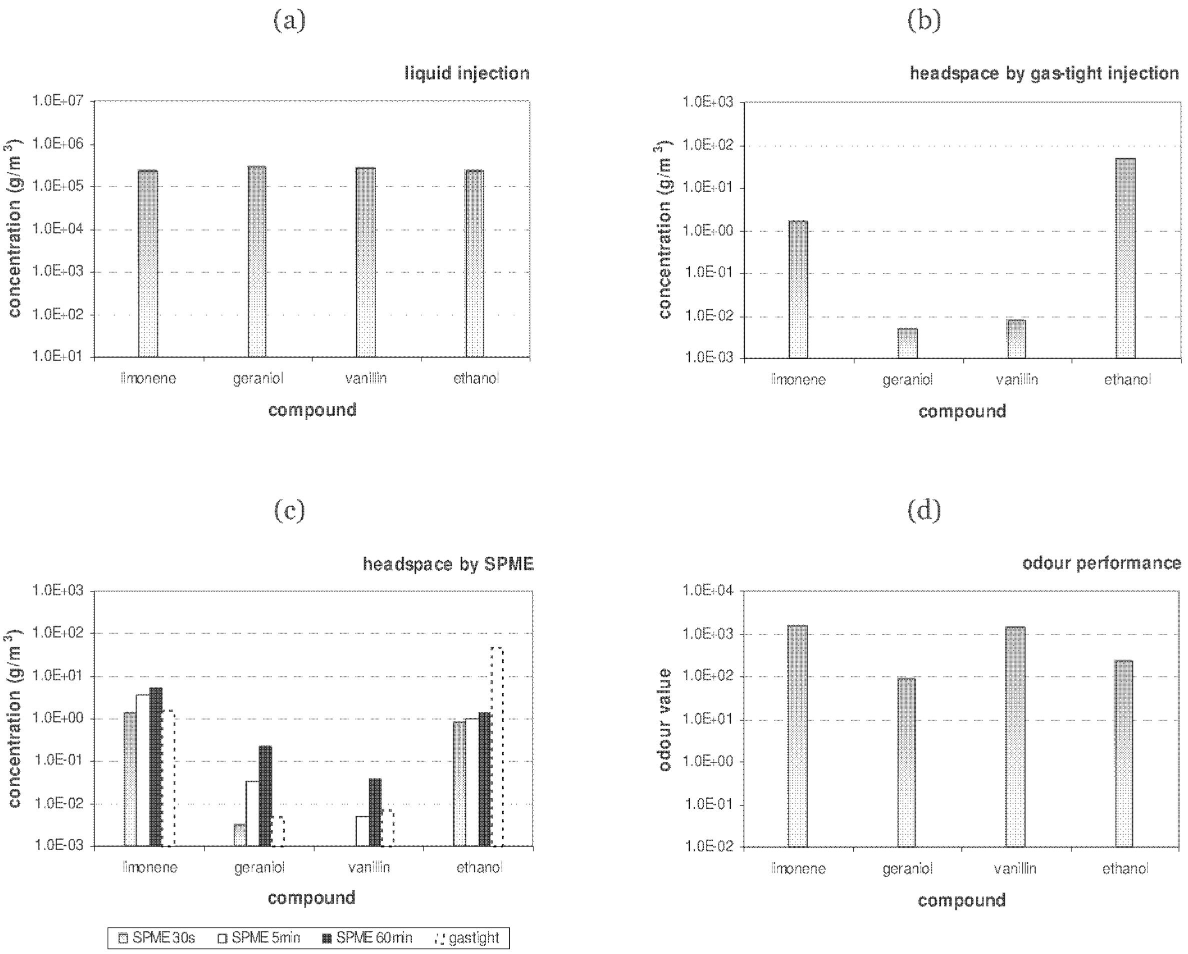


Figure 7.8 Composition profiles of the perfume test mixture at equilibrium and odour values at 25 °C: (a) liquid phase; (b) headspace by gastight injection; (c) headspace by SPME; (d) odour values.

direct injection using a gas-tight syringe in order to allow comparison of the results. For normalized concentration of 1, the headspace concentration determined by SPME is equal to the “true” concentration of the headspace, given by direct injection of headspace by gas-tight syringe (S-HS). If the normalized concentrations are below 1, the amount extracted is less than the one existing in the headspace. For values above 1, the SPME fiber is concentrating the component and displacing the equilibrium gas-liquid, by removing the vapour by adsorbing it into the fiber. In this case, the normalized concentration represents the concentration factor, as the number of times that the amount adsorbed is higher than the amount existing in the headspace.

It can be seen that the amount extracted by SPME changed considerably for the 4 components and with the time of exposure. The extraction was rather fast for the more volatile components (limonene

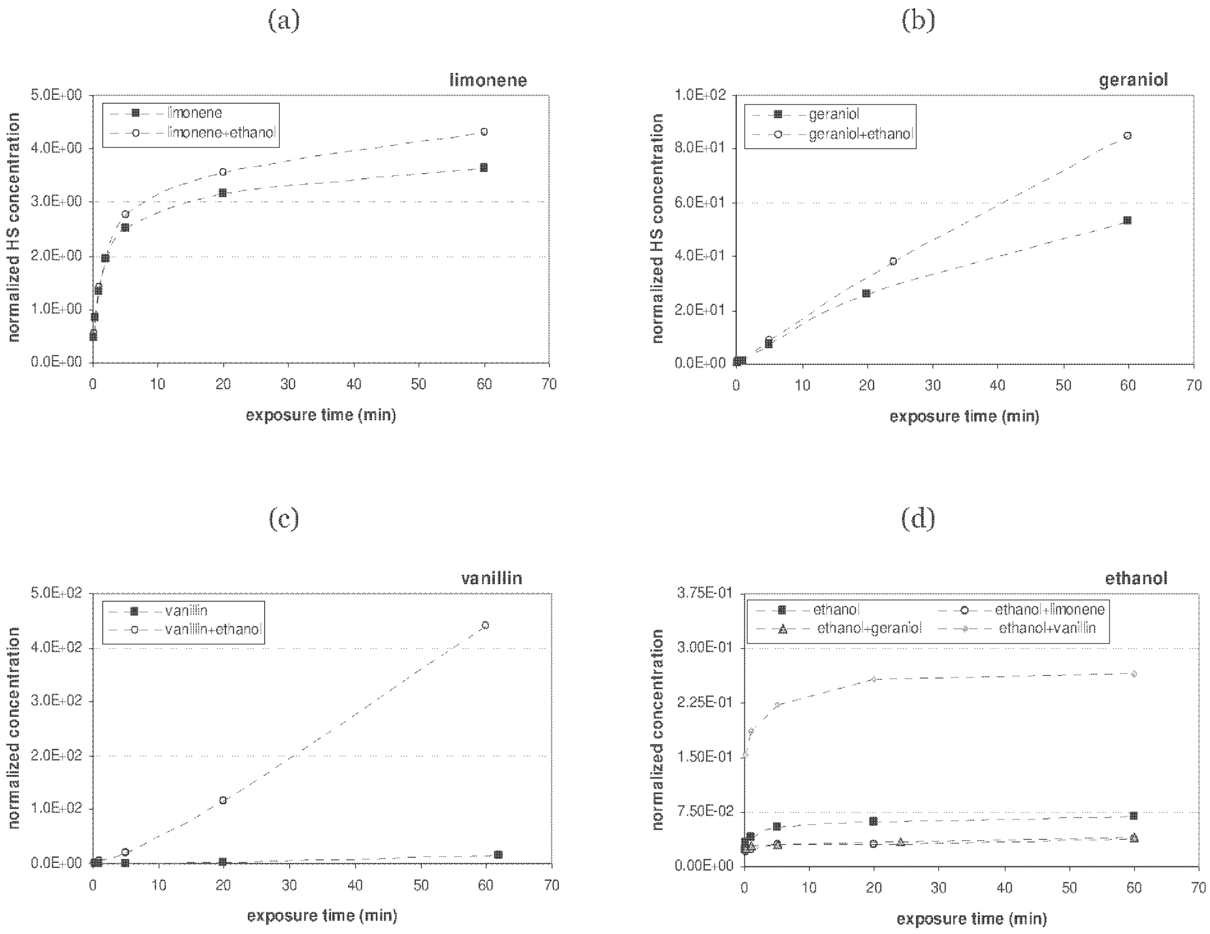


Figure 7.9 SPME extraction time profiles for the 4 components used in the perfume test mixture, pure and in a binary mixture with ethanol: (a)limonene; (b) geraniol; (c) vanillin and (d) ethanol.

and ethanol), with the profile becoming flat in the first minutes. For geraniol and vanillin, after one hour, the extracted amount was still increasing. On the other hand, the concentration factor was considerably higher for these two, since the normalized concentration is in the order of 10 for geraniol and 10^2 for vanillin. Moreover, it was found for ethanol that its normalized concentration was much smaller than 1 (in the order of 10^{-2}) even at exposure times of 15 s, which means that the SPME concentration was 100 times below the 'true' headspace concentration. We may conclude that the fiber was completely saturated and so it can not be used for the quantification of ethanol.

Comparing the extraction profiles for pure and binary mixtures, it is evident that the profiles change. The normalized concentration was higher for all the binary mixtures, limonene+ethanol, geraniol+ethanol and limonene+ethanol. This fact shows that a higher amount is adsorbed in the

SPME fiber, which means that the headspace concentration is higher and so apparently ethanol promotes the evaporation of the components. This effect was found to be greater for the less volatile components as shown by the biggest difference observed between the curves of pure component and binary mixture for vanillin.

Addressing all these considerations on both headspace techniques, by direct injection using gas-tight syringe (S-HS) and SPME, it was decided to use in this work the S-HS based on the fact that:

- the headspace concentrations are high enough to be measured by GC (they are above detection limit of the equipment);
- the method is simpler because the concentrations are calculated directly from GC peaks and calibration curves;
- there is no distortion in the concentration due to concentration and competition phenomena that occur in SPME technique;
- it is possible to quantify the solvent ethanol, which is not possible by SPME;
- the calibration of the SPME to determine the headspace concentration would require the preparation of standard gas solutions with known concentrations, what is very difficult to perform accurately; besides this, the calibration would only be valid at the very specific conditions at which they were determined and as the adsorption profile is very different for the 4 components, the competition phenomena are certainly significant.

7.3.3 Binary perfume mixtures

The Perfumery Ternary Diagram (PTD) methodology for predicting odour values was applied to a perfume test mixture, consisting of a ternary system of limonene (top note, A), geraniol (middle note, B) and vanillin (base note, C), in solution with ethanol (solvent, S). The simulations should be based on the perfumer olfactory sensitivity and therefore the OV values are recalculated using the values of odour thresholds obtained by panellist P1 (see Table 7.3). Also the equilibrium vapour pressures used were measured experimentally at the working room temperature of 25 °C (see Table 7.3).

To study the effect of non-ideality, solvent amount and adding another fragrant note on the OV values, binary mixtures will first be considered, in the same way that was done in Chapter 6.

Effect of non-ideality on OV

A binary liquid mixture of limonene and vanillin, A+C, was considered. This mixture is constituted by two fragrant chemical components having different volatilities and polarities, where A represents a non-polar top note and C a polar base note.

The variation of odour value for each component as a function of liquid composition in terms of molar fraction of C, $x_C = 1 - x_A$, for all the possible combinations A+C, is shown in Figure 7.10-a. It can be seen that this binary mixture is dominated by the odour of limonene for most of the compositions ($x_A > 0.005$), since $OV_{MAX} = OV_A$. Comparing the ideal and non-ideal case, the predicted OV considering non-ideal mixture are higher than OV obtained using the simplification of ideal liquid mixtures. The greatest deviation from ideality for limonene occurs at high molar fractions of vanillin, where the highly polar media 'pushes out' the non-polar molecules of limonene. The same effect is verified for vanillin at high molar fractions of limonene.

For the binary mixtures A+B (Figure 7.10-b), the deviations from ideality are less pronounced because geraniol is less polar than vanillin. It is found that limonene is again the dominant note, however geraniol becomes the most strongly noticed for compositions with $x_B > 0.92$, when $OV_{MAX} = OV_B$.

Figure 7.10-c shows a more balanced mixture in terms of OV, due to the fact that there are two polar molecules: the mixture has $OV_{MAX} = OV_B$ for compositions with $x_B > 0.5$. Also the deviations from ideality are considerably smaller. The OV values in this case are smaller for non-ideal solution because the two components show affinity for each other, being more retained in the liquid phase.

Effect of the solvent on the OV

The effect of the solvent in the odour value of each component can be seen in Figure 7.11. In this case, the solvent is ethanol (S) and the perfume a binary mixture of geraniol (B) and vanillin (C). The molar fractions are represented in a basis without solvent S, as described in Chapter 6. As ethanol is a polar solvent, the less-polar component (geraniol) will decrease its affinity to the medium with increasing molar fraction of the solvent. Consequently, the amount of B in the headspace is increasing and so its OV, and therefore the interception of the OV curves is moving towards higher amounts of C or less amounts of B. However, there is a point when the solvent becomes the component with the highest odour value, $OV_{MAX} = OV_S$. At $x_S = 0.5$, that happens for $x'_C > 0.38$ and at very high molar fractions of ethanol, $x_S = 0.9$, $OV_{MAX} = OV_S$ in practically all range of compositions.

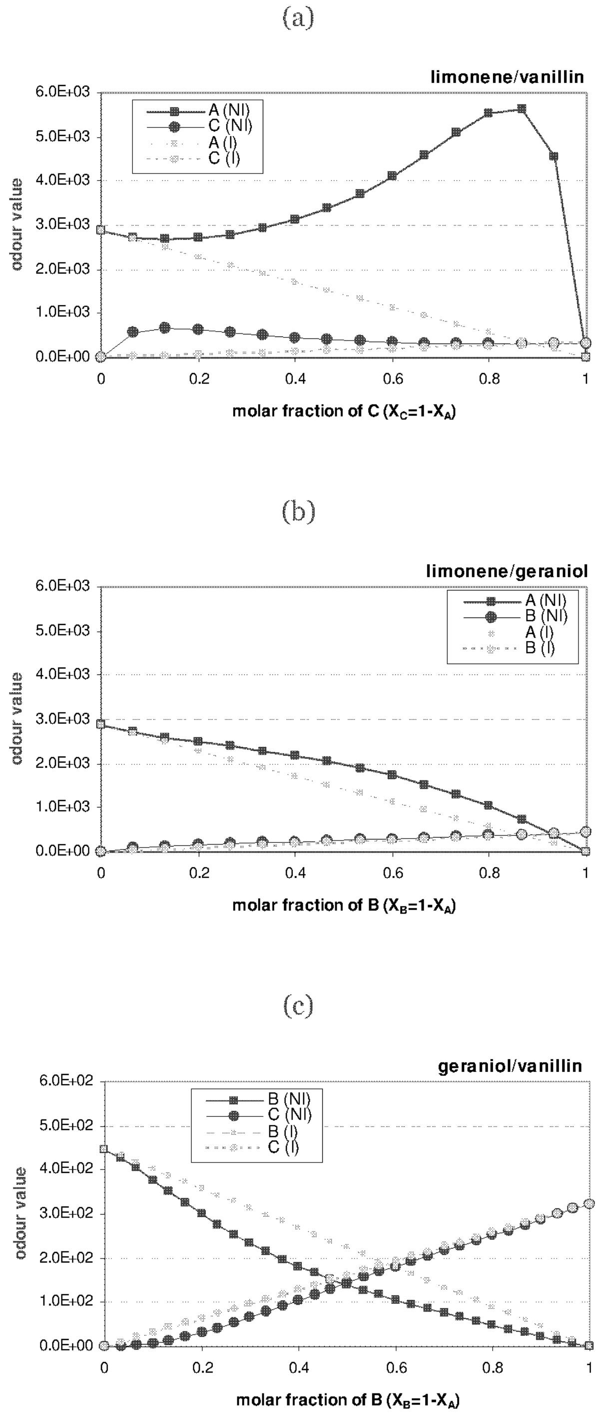


Figure 7.10 Comparison of odour value for the binary mixture: (a) limonene (A) and vanillin (C); (b) limonene (A) and geraniol (B); (c) geraniol (B) and vanillin (C). Considering the: ideal case (I) and non-ideal case (NI).

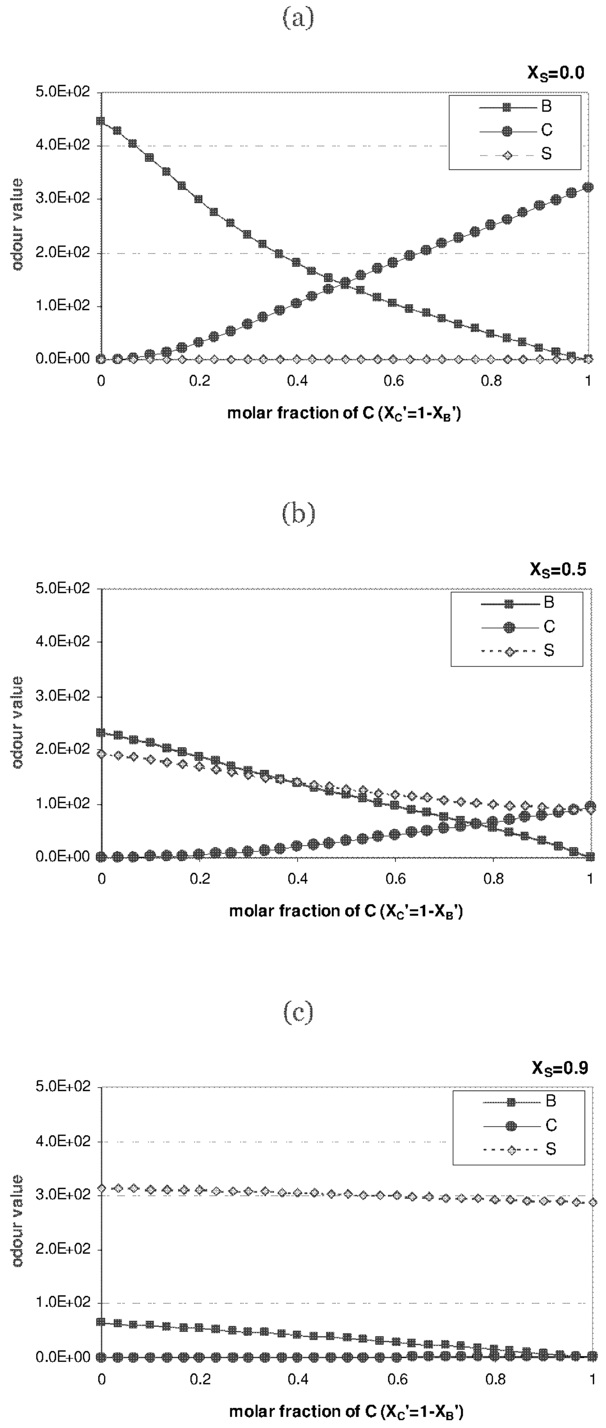


Figure 7.11 Effect of solvent (S) in the odour value of a binary mixture of geraniol (B) and vanillin(C): (a) Xs=0.0; (b) Xs=0.5 and (c) Xs=0.9.

Considering instead the effect of ethanol on the binary mixture A+C, shown in Figure 7.12, as limonene A is considerably more volatile, it remains the component with the highest OV over a wide range of compositions, even at high molar fractions of solvent. In this case, for $x_S = 0.9$, to assure that the strongest smell is that of limonene, i.e., $OV_{MAX} = OV_A$, it is enough to have $x'_A > 0.07$ ($x'_C < 0.93$). Otherwise, it will be ethanol having the highest OV. Note that for this perfume mixture, vanillin has never the maximum odour value.

The case of the effect of solvent on the mixture A+B is shown in Figure 7.13. As ethanol is a polar solvent, the non-polar component A (limonene) will decrease its affinity to the medium with increasing molar fraction of solvent. Therefore, the minimum amount necessary to make limonene the component with the highest OV is decreasing: $x'_A > 0.07$, when $x_S = 0$; $x'_A > 0.04$, when $x_S = 0.5$. However, when the solvent concentration is very high ($x_S = 0.9$), its headspace concentration is high enough to make $OV_{MAX} = OV_S$ at lower concentrations of limonene. In this case, a higher amount of A should be used, $x'_A > 0.11$, to make a mixture for which the highest OV is that of limonene (for $x'_A < 0.11$ ethanol has the highest OV). Note that for this diluted perfume mixture, geraniol has never the strongest odour.

Effect of adding a fragrant note on the OV

The effect of adding vanillin, C, as base note or fixative to the mixture of limonene+geraniol, A+B, is shown in Figure 7.14. It can be seen that an increase in C leads to an increase in OV_A and in a decrease in OV_B. The reason is because vanillin is a polar molecule and as its concentration increases, the media becomes more polar and so it tends to 'push out' the molecules of the non-polar component, limonene (A) from the liquid phase and, at the same time, to retain the more polar component, geraniol (B). In this case, adding a polar base note has the effect of stressing the strength of the top note, by increasing the headspace concentration of limonene, and to retain the polar middle note (geraniol), besides contributing itself to the overall odour.

In conclusion, the choice of the fixative is very important because it will determine both (i) the characteristic smell of the perfume and (ii) its persistence.

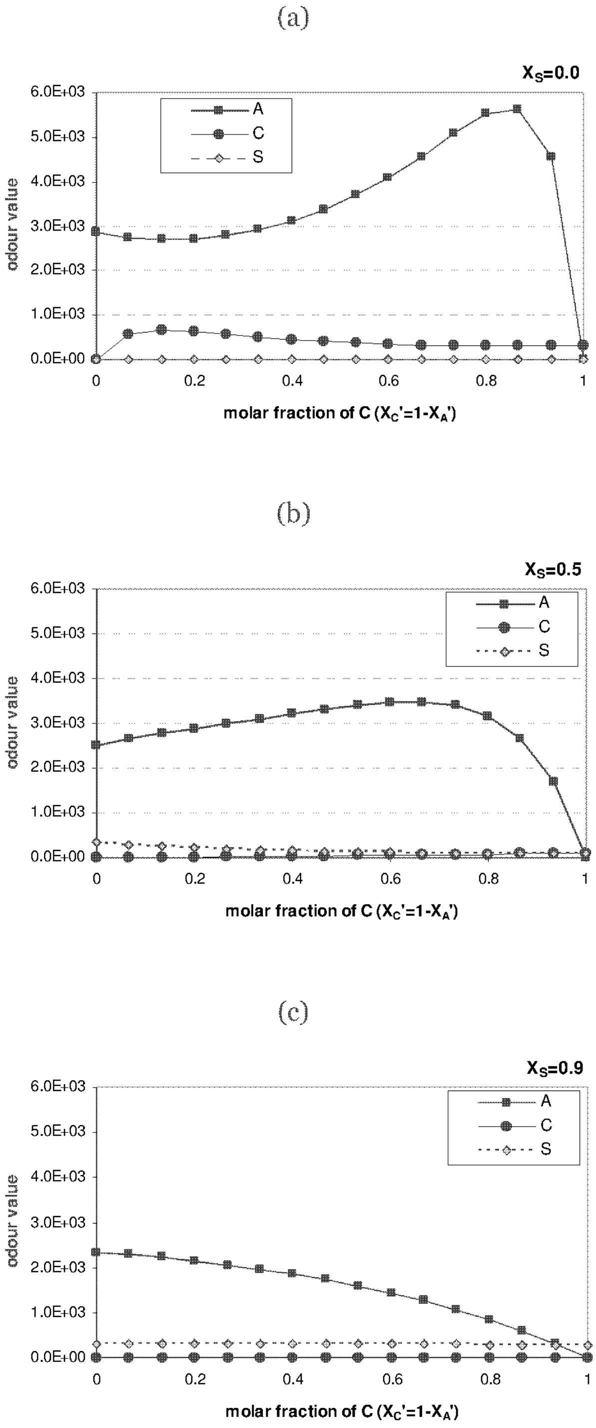


Figure 7.12 Effect of solvent (S) in the odour value of a binary mixture of limonene (A) and vanillin(C): (a) Xs=0.0; (b) Xs=0.5 and (c) Xs=0.9.

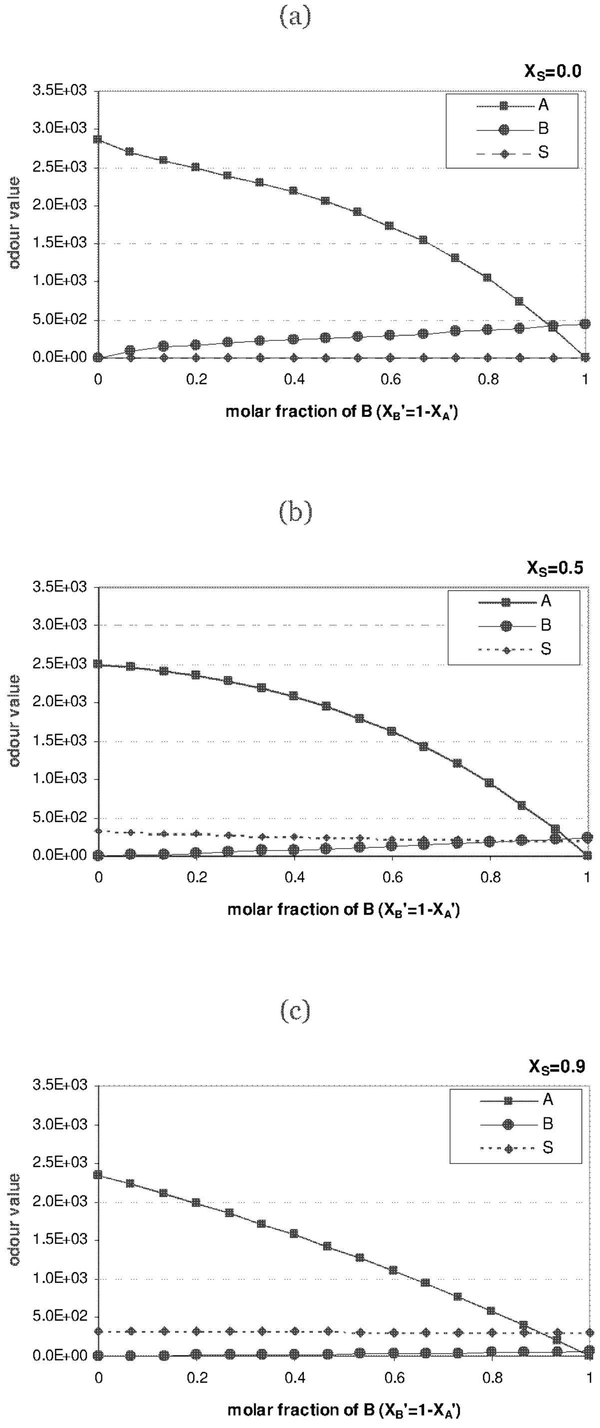
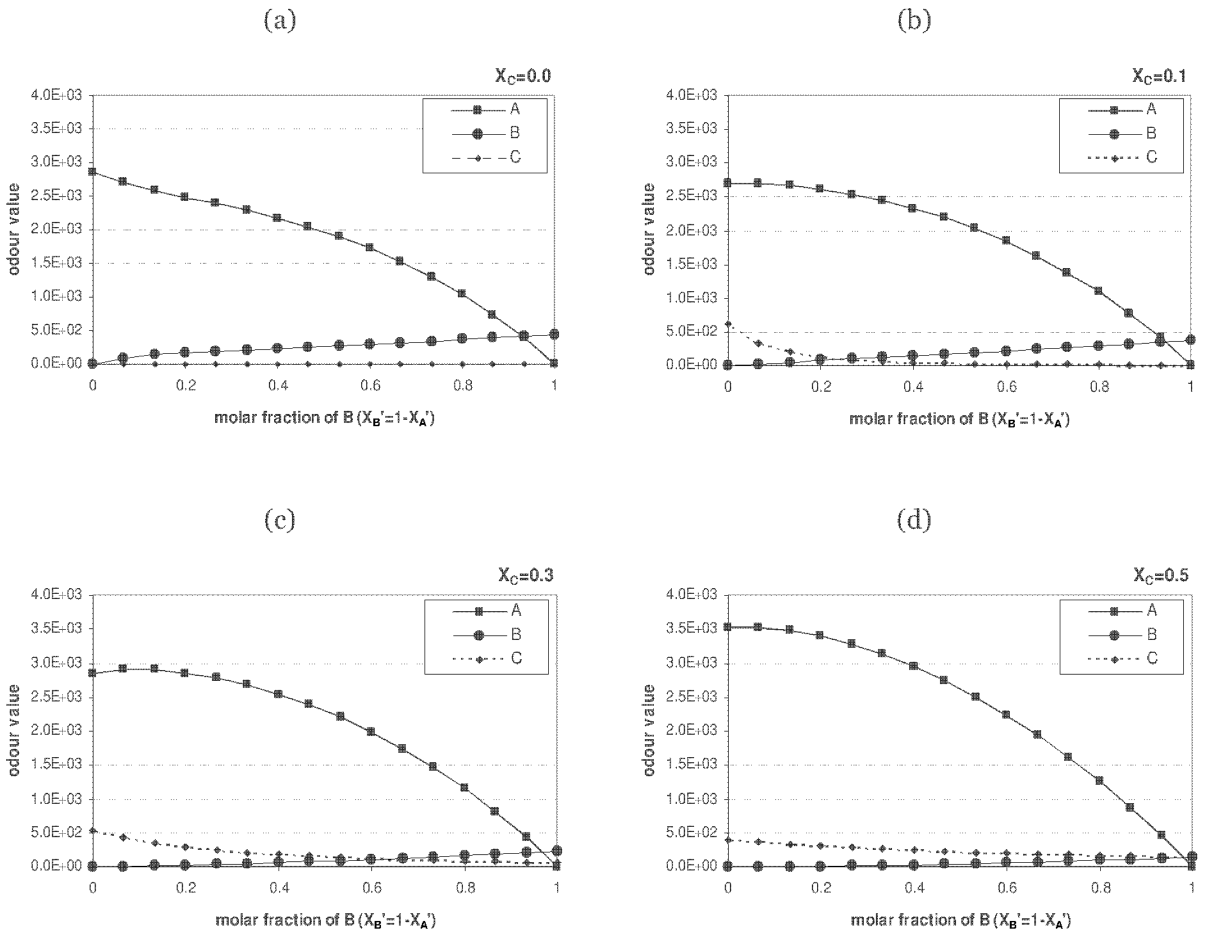


Figure 7.13 Effect of solvent (S) in the odour value of a binary mixture of limonene (A) and geraniol (B): (a) Xs=0.0; (b) Xs=0.5 and (c) Xs=0.9.


(a)

(b)

(c)

(d)

Figure 7.14 Effect of base note vanillin (C) in the odour value of a binary mixture of limonene (A) and geraniol (B): (a) Xc=0.0; (b) Xc=0.1; (c) Xc=0.3 and (d)) Xc=0.5.

In Figure 7.15, the case of adding a polar middle note B to a mixture A+C is shown. The effect of adding B is subtle, because the maximum OV continues to be that of limonene although its OV decreases, meaning that the odour should be less intense, for the same composition. The OV decreases since the medium becomes less polar with increasing amount of B, so the tendency of the media to 'push out' the non-polar A is diminished and its headspace concentration is smaller.

On the other hand, adding a top note A to the mixture B+C, has an overwhelming effect of the odour value of A overlapping to the odour values of B and C, even at small molar fractions of $x'_A < 0.1$, as can be seen in Figure 7.16.

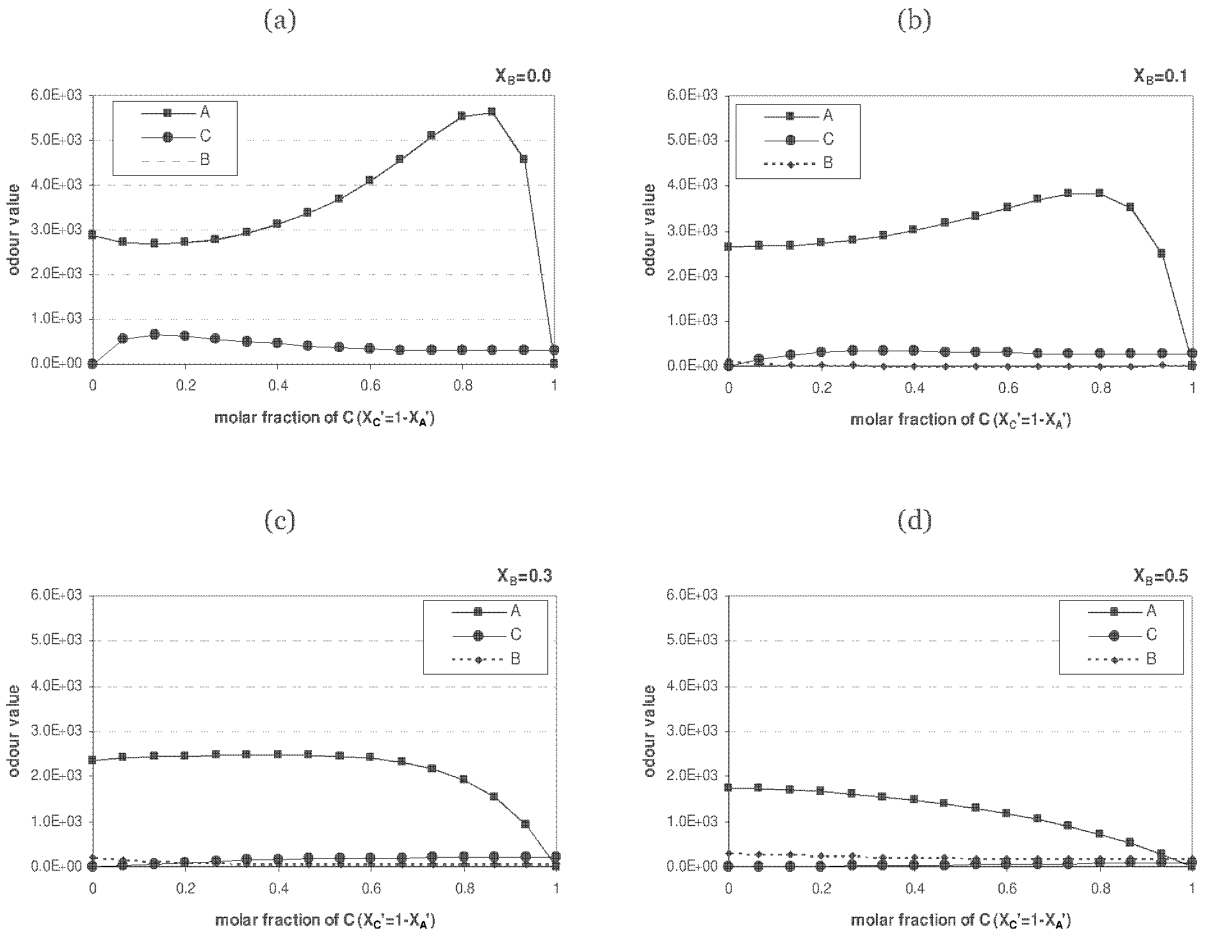


Figure 7.15 Effect of floral note geraniol (B) in the odour value of a binary mixture of limonene (A) and vanillin (C): (a) X_B=0.0; (b) X_B=0.1; (c) X_B=0.3 and (d)) X_B=0.5.

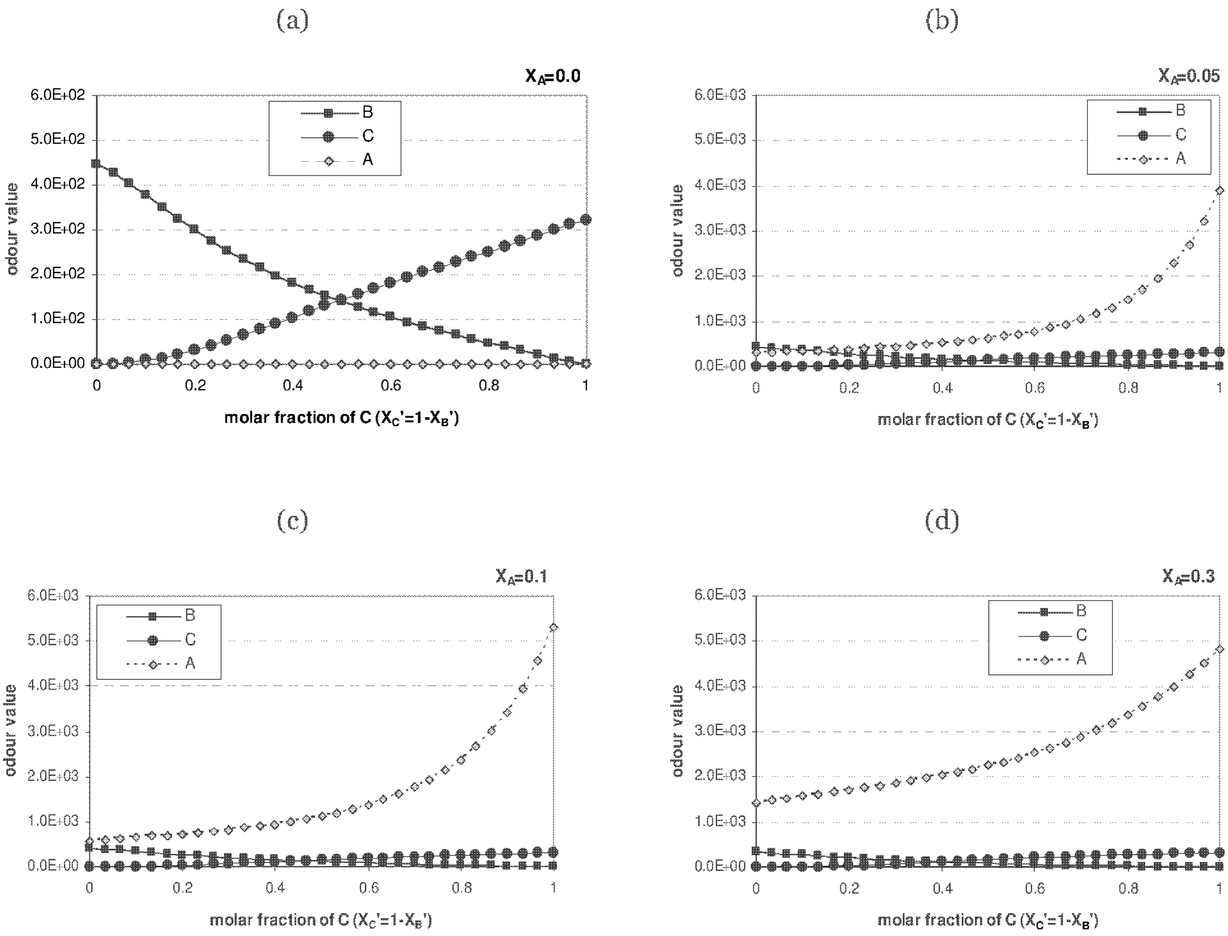


Figure 7.16 Effect of top note limonene (A) in the odour value of a binary mixture of geraniol (B) and vanillin (C): (a) X_A=0.0; (b) X_A=0.05; (c) X_A=0.1 and (d) X_A=0.3.

7.3.4 Perfumery Ternary Diagrams as a predictive tool

Considering now the ternary perfume mixture A+B+C in a solution in ethanol (S), the new PTD profiles for the ideal and non-ideal cases are shown in Figure 7.17 , with predicted OV values based on experimental data in Table 7.3. It can be seen that again this test perfume is mostly dominated by the limonene (A) top note, where geraniol (B) and vanillin (C) are only perceived at very low amounts of A in the ideal case and only after A has completely evaporated in the non-ideal case. Comparing with the PTDs obtained in Chapter 6, the profiles are very different owing to the fact that panellist P1 is rather more sensitive to limonene and less sensitive to geraniol and vanillin (compare values in Table 6.1 and Table 7.3), thereby there is a bigger region with $OV_{\max} = OV_A$.

The effect of the solvent on PTD based on experimental data is shown in Figure 7.18. Comparing with the PTDs showed in Chapter 6 (Figure 6.11), it can be seen that in this case (for panellist P1 sensitivity) the solvent has less influence in the OV value, where ethanol at an amount as high as $x_S = 0.7$ becomes noticeable only after limonene has completely evaporated (the values of $OV_{\max} = OV_S$ are located at the binary line B-C, corresponding to $x_A = 0$).

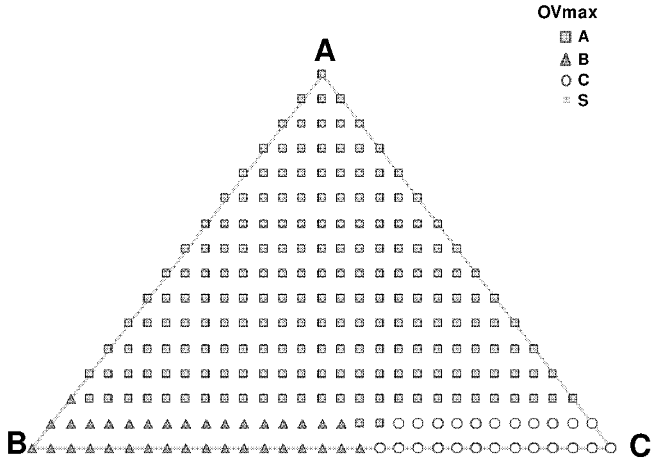
7.3.5 Validation of PTD methodology

The odour values determined based on data determined experimentally of headspace concentrations, vapour pressures and odour threshold values were compared with the odour values predicted using the Perfumery Ternary Diagram (PTD) methodology, for a perfume test mixture, as presented next.

The perfume test mixtures of limonene (A), geraniol (B) and vanillin (C), A+B+C, were prepared in ethanol, with the compositions in a basis without solvent, shown in Table 7.4. The composition points P1, P2 and P3 are also shown in the PTD in Figure 7.19. The molar fraction of ethanol was higher to the point P1, due to the higher content of vanillin (solid). However, this does not have implications on the OV of the mixture, as can be seen by comparing PTDs in Figure 7.18- b and c. All the compositions correspond to perfume test mixtures with limonene having the highest odour value ($OV_{\max} = OV_A$), although the amount of each component is considerably different. This was confirmed by smelling the headspace of the perfume test mixtures, which actually had the strongest odour of lemon (limonene, A).

(a)

IDEAL



(b)

NON-IDEAL

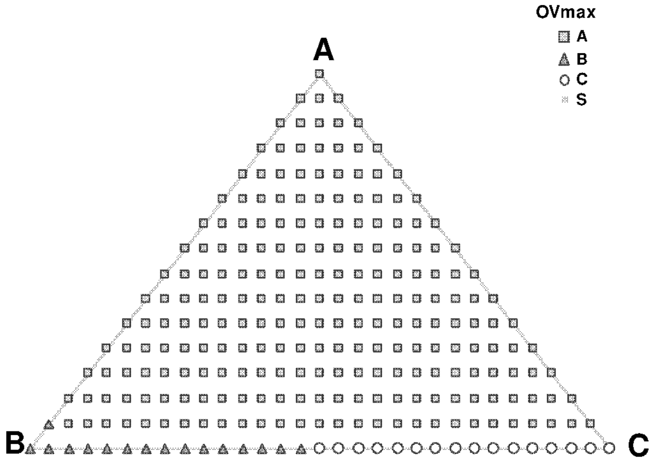
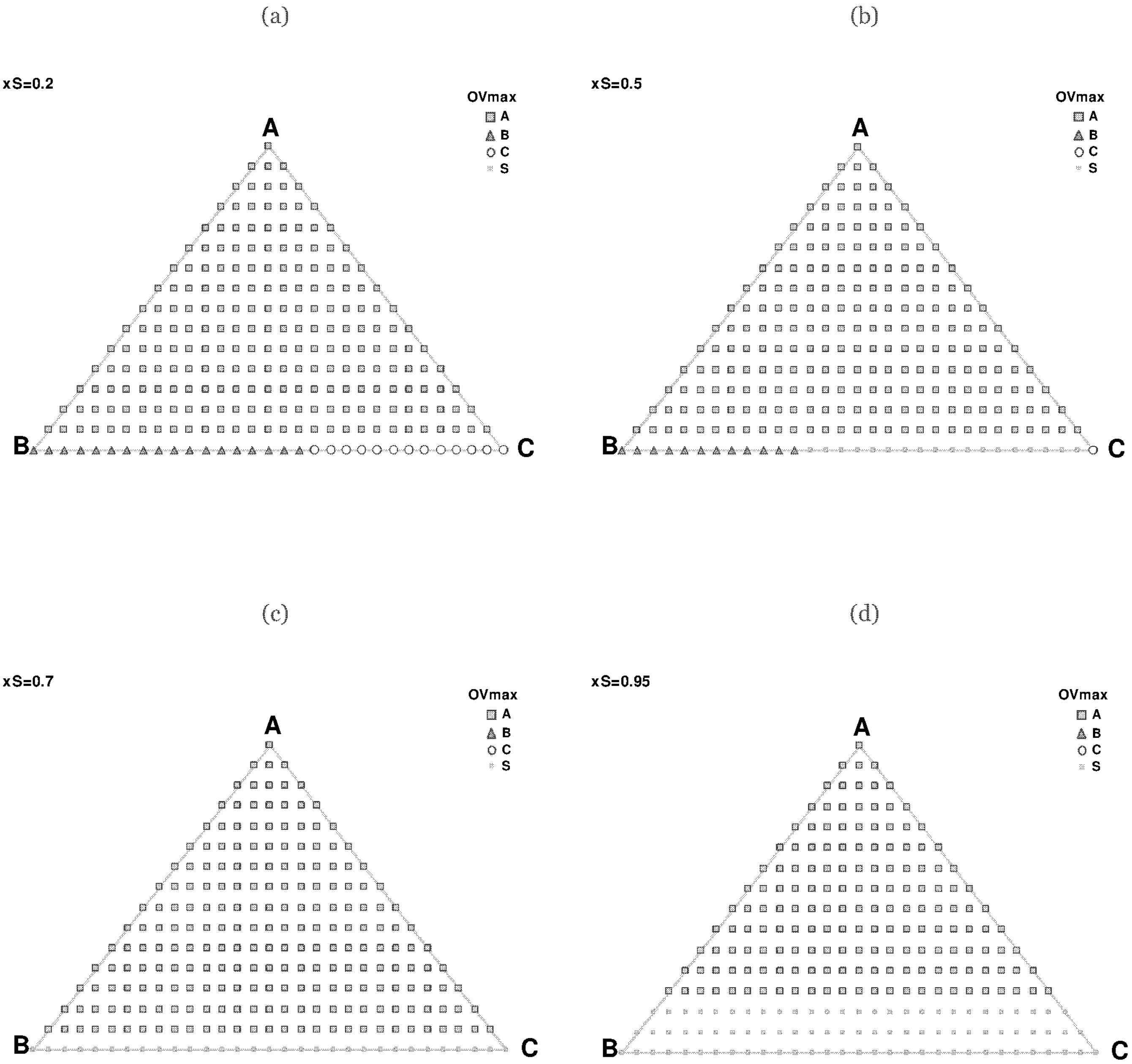


Figure 7.17 Perfumery Ternary Diagram of the perfume test mixture composed of limonene (A), geraniol (B) and vanillin (C): (a) ideal case; (b) non-ideal case.



(a) (b) (c) (d)

Figure 7.18 Effect of ethanol on PTD of a perfume test mixture composed of limonene (A), geraniol (B,) vanillin (C) and ethanol (S): (a) Xs=0.2; (b) Xs=0.5; (c) Xs=0.7; (d) Xs=0.95.

Table 7.4 Composition of perfume test mixtures used in the experiments, as molar fraction in a basis without solvent.

	Component	P1⁽¹⁾	P2⁽²⁾	P3⁽²⁾
A	limonene	0.333	0.599	0.199
B	geraniol	0.333	0.301	0.700
C	vanillin	0.334	0.100	0.100

(1) molar fraction of solvent used was 0.614;

(2) molar fraction of solvent used was 0.517

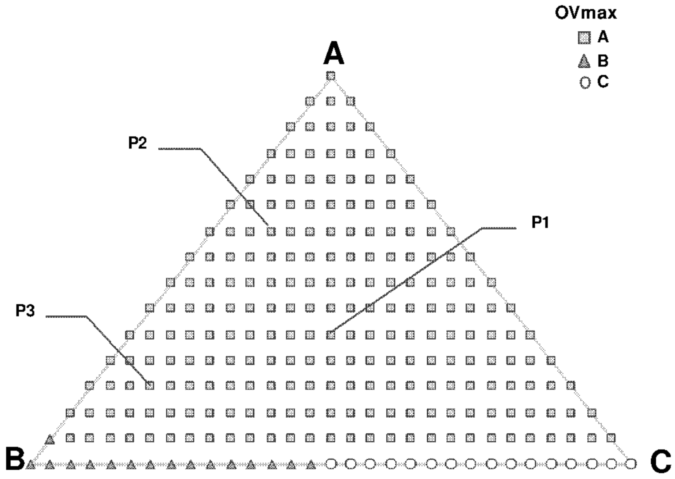


Figure 7.19 Perfumery Ternary Diagram and the composition points P1, P2 and P3 of a perfume test mixture with limonene (A), geraniol (B) and vanillin (C), in a basis without solvent ethanol (S), considering the non-ideal case.

The comparison of the headspace concentrations experimentally measured and the simulated headspace concentrations using PTD model, for ideal and non-ideal case, is shown in Figure 7.20. It was concluded that the behaviour of limonene and geraniol was acceptably predicted, being the headspace concentration determined experimentally closer to the simulated non-ideal case. However, for the very polar component, vanillin, the headspace concentrations obtained experimentally were quite different from the ones predicted by PTD model. The headspace concentration was higher for high amount of vanillin and limonene (P1 and P2), and lower for lower amount of limonene (P3). This means that the activity coefficients for vanillin were clearly underestimated in the case of P1 and P2 (non-polar or medium-polar media) and overestimated in the case of P3 (polar media). Considering compositions P1 and P2, the fact that the predicted values for non-ideal are lower than ideal case and in reality the experimental are higher than ideal case means that vanillin is actually being 'pushed out' from the solution instead of being retained has would be expected due to the affinity with geraniol. Therefore, the affinity vanillin-geraniol is overestimated, and in reality the repulsion limonene-vanillin is dominant. On the other hand, for composition P3, with low amount of limonene, the affinity geraniol-vanillin is dominant and therefore the headspace amount is lower than predicted in non-ideal and ideal cases. Finally, comparing the values simulated and measured for ethanol, it can be seen that the headspace concentration is higher than the ones predicted by PTD model, for all the compositions considered, being though closer to the simulated non-ideal values.

It is apparent from these results that exists a special interaction limonene-vanillin. In fact, vanillin is one of the most difficult perfumery materials to work with, although it is one of the most important, being part of nearly every perfume (Calkin and Jellinek, 1994). In concentrations as low as 0.5%, vanillin can overlay the odour of other components in a perfume composition. Therefore, it is usually the last ingredient to add and the most effective concentration level is established by trials.

The reason for the differences found between simulated values and experimental data can be related with the UNIFAC model used to predict the inter-molecular interactions and activity coefficients. In some cases, poor results are obtained using UNIFAC model and so new structural groups have to be introduced to improve the prediction of phase equilibria, namely acetals, amides, lactones, aromatic esters and aldehydes, and many others (Constantinescu *et al.*, 2001; Gmehling *et al.*, 2002; Teodorescu *et al.*, 2003). Thereby, the model behind PTD methodology may also be improved by introducing new structural groups, especially differentiating the cyclic from linear structure, in order

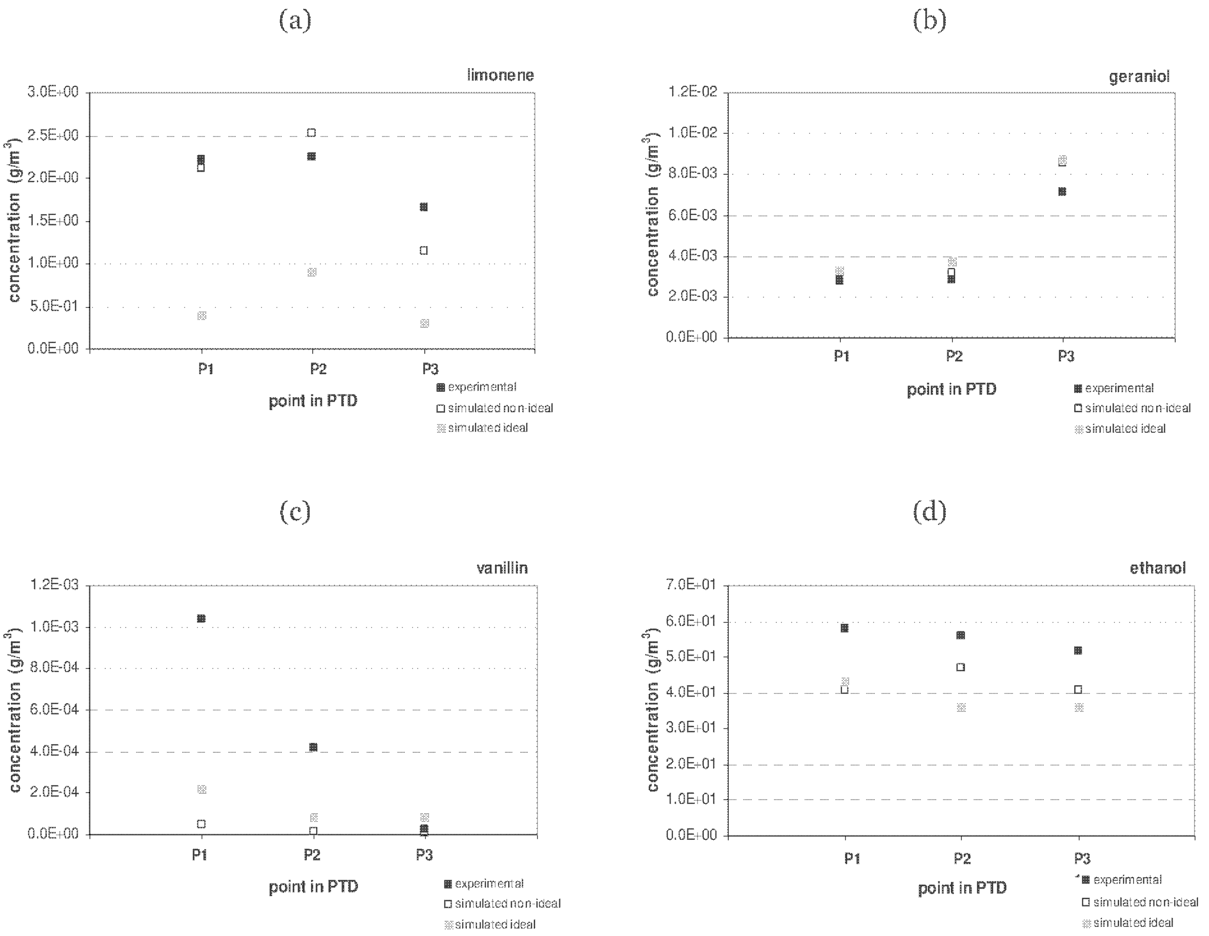


Figure 7.20 Comparison of experimental and simulated headspace concentration (ideal and non-ideal case), in equilibrium at 25 °C with the perfume test mixture with limonene, geraniol and vanillin in ethanol, for composition points P1, P2 and P3: (a) limonene; (b) geraniol; (c) vanillin; (d) ethanol

to represent better the complex nature of the fragrance materials. However, this task is beyond the scope of the objectives of this thesis.

7.4 Conclusions

Although the perfume is formulated in the liquid phase, it is the headspace concentration that will determine the smell of the composition. The odour value (OV) was the parameter used for the objective characterization of performance of a perfume test mixture, because it is based on measurable data. For this purpose, the odour threshold values, vapour pressures and headspace concentrations were determined experimentally.

The odour threshold values in air of several aroma chemicals were determined using an olfactometer, at 25 °C.

The headspace SPME analysis, depending on the sampling time, can be more or less representative of the concentration in the headspace. In these conditions, SPME can not be used to quantify limonene and ethanol, since the fiber was already saturated in these components after 30 s of sampling time. On the other hand, the fiber concentrates 100 to 1000 times the amount of geraniol and vanillin existing in the headspace. This means that the equilibrium gas-liquid was displaced and that a higher quantity of geraniol and vanillin was evaporated. Moreover, as vanillin has a lower volatility than geraniol, the increase of adsorbed mass was higher for geraniol.

It was shown that the headspace analysis by direct injection using a gas-tight syringe was preferable because there is minimum distortion of the headspace concentration, except in the case of very low amounts, which are below the detection limits of the analysis equipment (GC).

The Perfumery Ternary Diagrams (PTD) profile obtained for the perfume test mixture composed of limonene, geraniol and vanillin, A+B+C, changed significantly from the one using the data found in the literature (Chapter 6), mainly due to the differences verified between the experimental values and the literature data for odour threshold values.

Comparing the odour values (OV) predicted by PTD model and the ones obtained based on experimental data, it was concluded that the OV for the non-polar component (limonene) and medium-polar component (geraniol) was reasonably well predicted, while the OV for the polar components (vanillin and ethanol) were poorly estimated, showing that the UNIFAC model should be improved by introducing new structural groups that take into account the complexity of the fragrance molecules, which include cyclic components with many functional groups, or use other models such as Modified UNIFAC or NRTL (Reid *et al.*, 1987). Nevertheless, the methodology has proven to be useful in predicting OV values of perfume mixtures, giving some insight on the behaviour of the components in solution, and how that affects the headspace composition and its odour.

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CHAPTER 8

CARACTERISATION OF ODOUR PERFORMANCE USING A PERFUME DIFFUSION MODEL

A diffusion model was used to simulate the evaporation of a small multi-component and finite sample of perfume test mixture. This model considers the liquid as a non-ideal solution and takes into account the changes of composition and volume during the evaporation of small volumes of liquid. The changes in the liquid phase and in the gas-liquid interface were followed using the new concept of Perfumery Ternary Diagrams (PTD). A characteristic point of the PTD was used as the initial mixture for simulating the evaporation of the small amount of perfume as a function of time and space. The performance parameters: impact, tenacity, diffusion and volume, were discussed and quantified based on these results.

8.1 Introduction

The fragrance performance is especially important when considering functional products, including all the stages of their application. For example, it is important that a fabric softener imparts a pleasant smell during washing, after drying and during storage in the closet. Therefore there is an optimal amount to use, below which the client/consumer will not perceive it and if it is higher the client/consumer can refuse to buy it for being unpleasantly intense or for having a high cost (Calkin and Jellinek, 1994). Besides, low volume means less impact on health and on the environment (Rouhi, 2003).

Perfumers have evaluated fragrance performance in empirical terms over many decades and described only qualitatively over time and location. Different aspects of performance can be distinguished (Calkin and Jellinek, 1994) as can be seen in Figure 8.1.

Impact refers to the efficacy of a perfume during the first moments of product performance, near the application point; for example, a top note is noticed immediately when sniffing at the perfume bottle or some seconds after applying the product to the skin. Diffusion is a measure of the distance over which the fragrance is noticeable soon after application; for example, high diffusion is desirable, for example, in a bath foam or a dishwashing detergent. Tenacity refers to the long-term effectiveness of the fragrance in the perfumed product, in the proximity of the application area, for example, upon the skin after spraying a perfume or using a soap. Volume is the effectiveness over distance, some time after application.

The making of a quality perfume (also named compounding) relies on the ability to select the right composition for the intended use or aim, and that still relies upon the long experience of the perfumer, having though a significant basis of empirical knowledge and trial-and-error. So the evaluation of the perfume quality and performance is very subjective because it depends on a judgment of the perfumer or evaluator, without any support on directly measurable data obtained by quantitative analytical methods. On the other hand, there are other factors that come into play, especially the fact that they refer to a sensory experience, which is a result of a complex superposition of individual physical phenomena occurring simultaneously, which are even known to depend on each person and finally depends on the person's individual sensitivity to register a scent perception (Gygax and Koch, 2001).

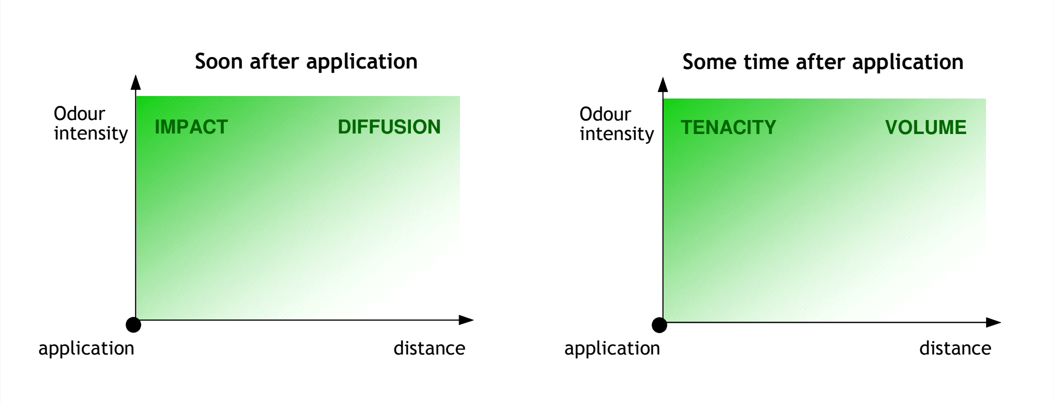


Figure 8.1 Graphical representation of the odour performance parameters:impact, diffusion, tenacity and volume.

Nevertheless, there are some systematic approaches based on physical properties, which are measurable parameters that can be used to impart some objectivity into perfume characterization and compounding, namely vapour pressure, water-solubility, octanol-water partition coefficients and activity coefficients. Other measurable parameters are odour threshold values and concentration in the vapour phase or headspace, which in turn depends on volatility, polarity and affinity to the liquid media. However, these data can be used to characterize only an isolated physical phenomenon and does not give complete information about a complex fragrance application process (Gygax and Koch, 2001).

The concept of Odour Value (OV), defined as the ratio between the concentration of that odourant component in the headspace – gas above the liquid mixture - and its odour threshold (Calkin and Jellinek, 1994), combines perfumery experience based on perception with objectively measurable parameters (Gygax and Koch, 2001). Note that although OV is based on concentrations, this parameter it is not additive, because it is related with odour intensity and this is not an additive parameter (Appell, 1969).

In this chapter, a mathematical model for perfume evaporation was used in order to predict the behaviour of one perfume as a function of time and space. The same model is used to characterize and quantify the odour performance parameters of one perfume, as it will be seen next.

8.2 Description of diffusion model

A volume of liquid perfume that is evaporating in time and diffusing upwards through the air above it – headspace - constitutes the following developed perfume model (Figure 8.2). It is considered that the liquid composition changes with time because a small amount of perfume is used. The composition of this liquid is represented by the number of moles of each species, n_i, and the headspace composition is represented by the concentration of each component, c_i in the total air volume considered, V.

It is assumed that diffusion takes place in only one direction (z) and that the surrounding air is not soluble in the perfume mixture.

The following conditions for gas phase, liquid phase and initial and boundary conditions are assumed.

8.2.1 Gas phase

The gas phase is considered to be in a bottle of cylindrical shape and constant volume, where the perfume molecules diffuse in only one direction, z.

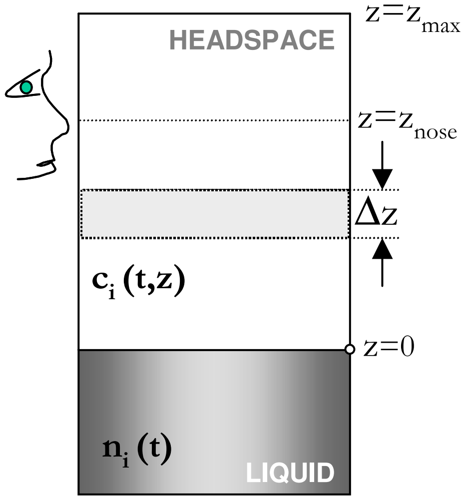


Figure 8.2 Scheme of the simulated gas (headspace)/liquid perfume system

The solute mass in the gas phase is the result of a mass balance in the gas control volume $\Delta V = A\Delta z$, where V is the total volume of the gas phase and A is the interfacial gas-liquid area. Considering that the total volume of the gas phase V, is constant, the gas phase is an ideal solution and that temperature and pressure are also constant, the unsteady state mass balance can be represented by the Fick's second law:

$$\frac{\partial y_i}{\partial t} = D_{i,air}\frac{d^2 y_i}{dz^2} \tag{8.1}$$

where y_i is the mole fraction of species i in the gas phase, t is the time, z is the vertical distance to the interface gas liquid and $D_{i,air}$ is the diffusivity of solute i in the gas mixture. The value of $D_{i,air} = D$ was calculated using the method of Fuller *et al.* (Reid *et al.*, 1987) (see Appendix B). This equation gives the concentration profile, i.e., the variation of concentration with position at a fixed time. The value of mole fraction of species i in the gas phase is given by $y_i = c_i/c_T$, where c_i is the concentration of species i in the gas phase and c_T is the total gas concentration, calculated as $c_T = {}^{P}\!/_{RT}$, which is constant.

8.2.2 Liquid phase

It is considered that the concentration and volume of the liquid phase changes with time t, as the multi-component mixture vaporizes, and that the liquid solution is uniform for each value of t (perfectly stirred solution). The release of fragrant components (solutes) occurs due to the mass transfer from the liquid into the gas phase by diffusion, through the interfacial layer, as follows:

$$\frac{\partial n_i}{\partial t} = -DAc_T\left.\frac{dy_i}{dz}\right|_{z=0} \tag{8.2}$$

where n_i is the number of moles of component i in the liquid.

8.2.3 Boundary and initial conditions

Equation 8.1 is subjected to the following initial conditions:

$$\text{Gas: } t = 0 : y_i = y_{i0} = 0 \tag{8.3}$$

$$\text{Liquid: } t = 0 : n_i = n_{i0} \text{ or } x_i = x_{i0} \tag{8.4}$$

where n_{i0} is the number of moles of component i in the initial perfume mixture and x_{i0} is the initial molar fraction of component i in the same initial perfume mixture.

For $t > 0$, it is assumed that the liquid phase is in equilibrium with the gas phase in contact with the interface $(z = 0)$. The following boundary conditions were used:

Gas:

$$\begin{aligned} &z = 0 : y_i = y_{i0} = \frac{\gamma_i P_i^{sat}}{P} x_i = \frac{\gamma_i P_i^{sat}}{P} \frac{n_i}{\sum n_i} \\ &z > 0 : y_i = y_{i0} = 0 \end{aligned} \tag{8.5}$$

Liquid:

$$z = 0 : n_i \big|_t = n_i \big|_{t-1} - ADc_T \int_{t-1}^{t} \left. \frac{\partial y_i}{\partial z} \right|_{z=0} dt \tag{8.6}$$

where y_{i0} is the gas mole fraction of component i at the interface gas-liquid in equilibrium with component i in the liquid phase with mol fraction x_i.

8.2.4 Numerical solution

Model equations were numerically solved by using the software package PDECOL (Madsen and Sincovec, 1979) based on the method of lines and uses orthogonal collocation in finite elements (OCFE) for the discretization of the space variable. For a ternary system there are six PDEs, which are discretized in the axial direction using 20 finite elements and two interior collocation points in each element; the resulting system of ODE's in the time variable is integrated with the solver GEARIB (Hindmarsch, 1976). The tolerance used was 10^{-8}.

For high volumes of liquid perfume phase, it is considered that the number of moles in the liquid phase is almost constant, i.e., $n_i \cong n_{i0}$. This implies that the concentration in the gas phase at the gas-liquid interface, c_{i0} is also constant. In this case, Equation 8.1 has an analytical solution, by defining a new variable, $\varsigma = \frac{z}{\sqrt{4Dt}}$ (Cussler, 1997), resulting in:

$$\frac{y_i - y_{i,gl}}{y_{i,\infty} - y_{i,gl}} = \mathrm{erf}(\varsigma)\,, \tag{8.7}$$

where

$$\mathrm{erf}(\varsigma) = \frac{2}{\sqrt{\pi}} \int_0^{\varsigma} e^{-s^2}\, \mathrm{d}s \tag{8.8}$$

One example in perfumery where this situation can occur is during the evaporation of perfume from its bottle. In this case, the volume is high and evaporation is almost negligible, thus the concentration in the liquid phase is almost constant.

8.3 Case study

The model was tested using a four-component mixture, representing one typical perfume. Again, this mixture is constituted by limonene (A), geraniol (B) and vanillin (C) and the solvent ethanol (S). The perfume test mixture is represented by a finite and small volume of liquid perfume, which is continuously changing with time.

8.3.1 Caracterisation of the case study system

Table 8.1 shows the physical variables that are important for the studied evaporation model for each component. The initial liquid compositions, x_{i0} for $i = A, B, C$ and the corresponding values of the liquid activity coefficients, γ_i, the gas molar fraction at the interface gas-liquid in equilibrium with x_{i0}

using Equation 6.4, y_i and the odour value of the headspace at this interface point, OV_i, for ten initial perfume composition points: P1-P10 that are presented in Table 8.2.

Points P1 through P4 represent the initial perfume composition values (expressed as molar fractions) used to simulate the evolution of OV during evaporation, named evaporation paths, which are shown in Figure 8.3 and Figure 8.4. These points are represented in the PTD of Figure 8.5. Points P5 through P9 are the initial perfume composition values used to simulate the corresponding evaporation paths, shown in Figure 8.6. These points are represented in the PTD of Figure 8.7. Finally, point P10 is the initial composition value for Figure 8.8 and it is represented in the PTD of Figure 8.8.

For all points, it was assumed an initial amount of test mixture, $n_{iT} = \sum n_i$ =1 mmol. The area of the interface gas-liquid, $A_{gl} = 0.071$ m^2, the pressure, $P = 10^5$ Pa (1 bar) and the temperature, $T = 298.15\,\text{K}$ (25°C).

8.3.2 Effect of the top note

Figure 8.3 shows the effect of the amount of the top note, A, in the evaporation of the test perfume mixture, corresponding to points P1 through P4 represented in Figure 8.5, for which the initial molar fraction of A, x_{A0} decreases and the molar fractions of B are equal to the molar fraction of C, $x_B = x_C$ (see Table 8.2). In all cases, the odour value of A is the maximum value, $OV_A = OV_{MAX}$, during the first minutes, respectively: for point P_1 this happens during 69.6 min, for point P_2 during 49.2 min, for point P_3 during 26.4 min and for point P_4 during 1.0 min. In all cases, after these times, $OV_C = OV_{MAX}$ and component B has never a maximum odour value.

After component A has almost disappeared, it remains a solution of almost B and C. This solution is very stable, as we can see in Figure 6.4-a, where the odour values obtained for non-ideal solutions are

Table 8.1 Parameters used in the diffusion model.

.	**Compound**	**Molecular formula**	**M** (g/mol)	C_{thr_i} (1) (g/m³)	P_i^{sat} (1) (Pa)	$D_{i,air}$ (m²/s)
A	limonene	$C_{10}H_{16}$	136.1	2.45×10^{-3}	2.05×10^{2}	6.15×10^{-6}
B	geraniol	$C_{10}H_{18}O$	154.3	2.48×10^{-5}	2.67×10^{0}	5.94×10^{-6}
C	vanillin	$C_8H_8O_3$	152.2	1.87×10^{-7}	1.60×10^{-2}	11.42×10^{-6}
S	ethanol	C_2H_6O	46.0	5.53×10^{-2}	7.27×10^{3}	10.68×10^{-6}

(1) data from Calkin and Jellinek (1994)

Table 8.2 Initial compositions for the gas-liquid case study perfume mixture.

Point	x_{A0}	x_{B0}	x_{C0}	γ_A	γ_B	γ_C	y_{Ao}	y_{Bo}	y_{Co}	OV_A	OV_B	OV_C
P1	0.800	0.100	0.100	1.171	0.904	7.235	1.90×10^{-3}	2.38×10^{-6}	1.14×10^{-7}	4.32×10^{3}	6.05×10^{2}	3.81×10^{3}
P2	0.667	0.167	0.167	1.399	0.625	3.860	1.89×10^{-3}	2.74×10^{-6}	1.02×10^{-7}	4.30×10^{3}	6.98×10^{2}	3.39×10^{3}
P3	0.467	0.267	0.267	1.928	0.503	2.040	1.82×10^{-3}	3.53×10^{-6}	8.59×10^{-8}	4.14×10^{3}	8.98×10^{2}	2.86×10^{3}
P4	0.200	0.400	0.400	3.186	0.522	1.176	1.29×10^{-3}	5.49×10^{-6}	7.43×10^{-8}	2.93×10^{3}	1.40×10^{3}	2.48×10^{3}
P5	0.733	0.200	0.067	1.224	1.133	3.251	1.82×10^{-3}	5.96×10^{-6}	3.42×10^{-8}	4.13×10^{3}	1.52×10^{3}	1.14×10^{3}
P6	0.733	0.167	0.100	1.251	0.914	4.297	1.86×10^{-3}	4.01×10^{-6}	6.78×10^{-8}	4.23×10^{3}	1.02×10^{3}	2.26×10^{3}
P7	0.733	0.133	0.133	1.275	0.728	5.136	1.89×10^{-3}	2.55×10^{-6}	1.08×10^{-7}	4.31×10^{3}	6.50×10^{2}	3.60×10^{3}
P8	0.733	0.100	0.167	1.296	0.568	5.756	1.93×10^{-3}	1.49×10^{-6}	1.51×10^{-7}	4.38×10^{3}	3.80×10^{2}	5.05×10^{3}
P9	0.733	0.067	0.200	1.314	0.429	6.187	1.95×10^{-3}	7.52×10^{-7}	1.95×10^{-7}	4.44×10^{3}	1.91×10^{2}	6.51×10^{3}
P10	0.200	0.433	0.367	3.006	0.555	1.134	1.22×10^{-3}	6.33×10^{-6}	6.57×10^{-8}	2.77×10^{3}	1.61×10^{3}	2.19×10^{3}

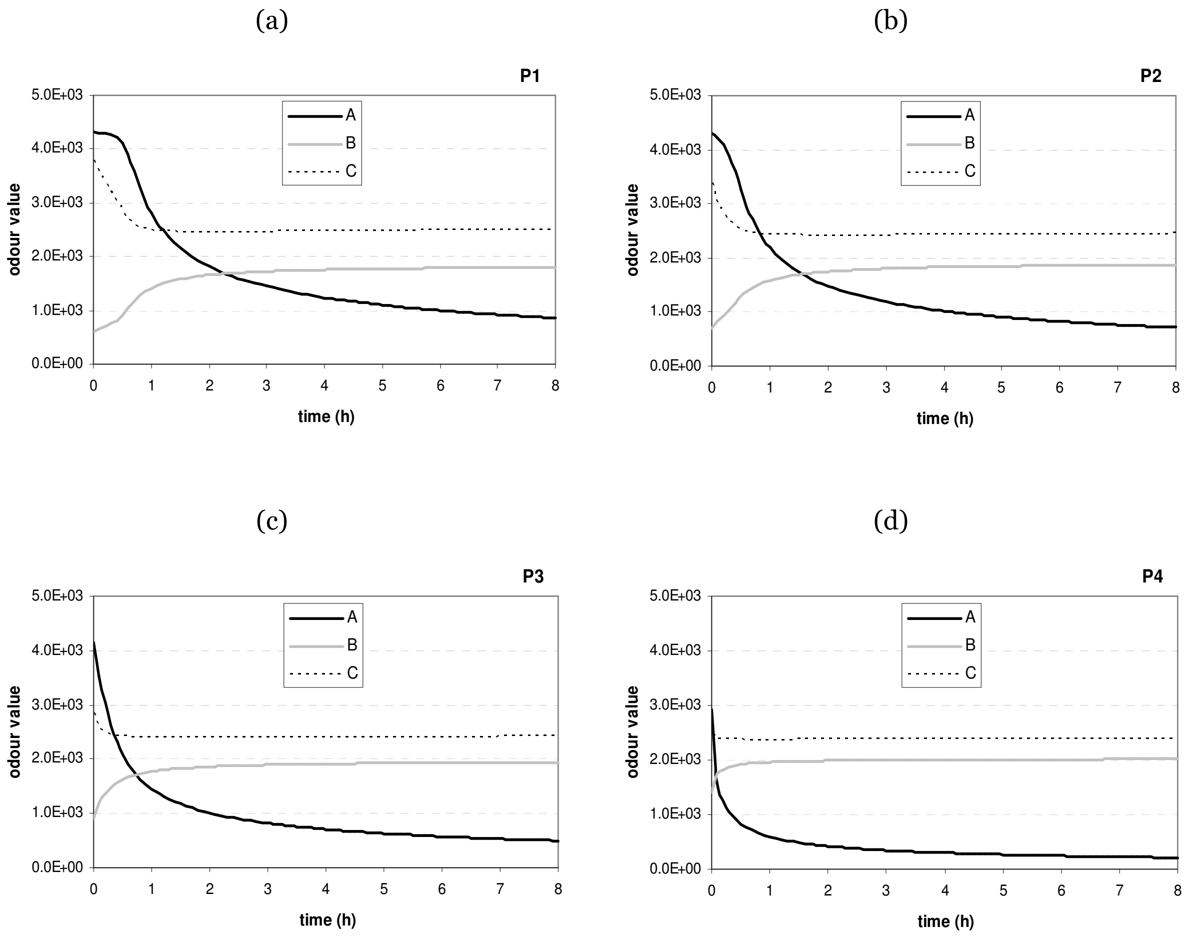


Figure 8.3 Variation of odour value as a function of time for different initial compositions shown in Table 8.2, with limonene (A), geraniol (B) and vanillin (C): (a) P1; (b) P2; (c) P3; (d) P4.

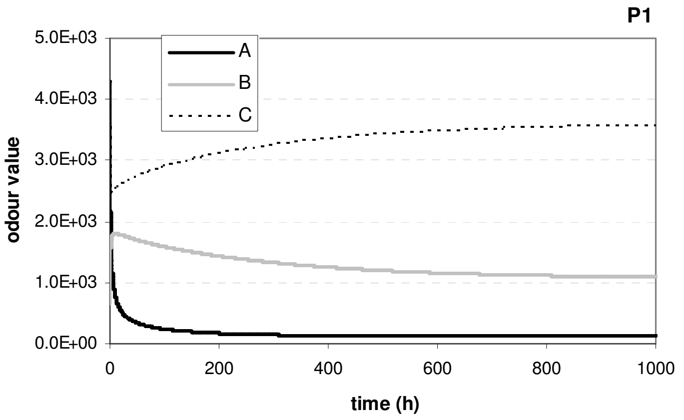


Figure 8.4 Variation of odour value as a function of time using an initial composition represented by point P1 for a long time, with limonene (A), geraniol (B) and vanillin (C).

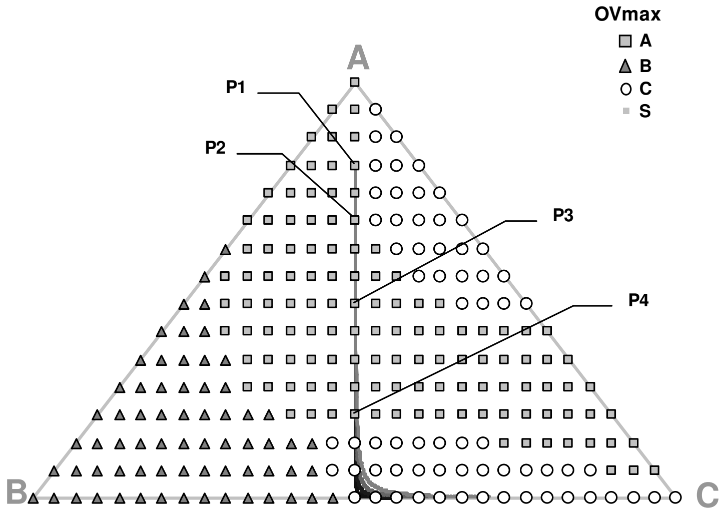


Figure 8.5 Evaporation paths represented in the perfumery ternary diagram of the test mixture, for the starting compositions P1, P2, P3 and P4, with limonene (A), geraniol (B), vanillin (C) and ethanol (S).

smaller than those obtained for the corresponding ideal solution. There exist two polar molecules, that tend to retain each other in the solution (γ_B and γ_C are less than 1 after component A has become in a smaller amount) and their evaporation is very slow, because they act as fixatives of each other. Figure 8.4 shows, in a log time scale, the variation of odour value, after some days. Component B disappears faster because its vapour pressure is higher, and the solution tends to pure C, the corner of the PTD. It can also be seen that the evaporation never passes through a point where the B has a maximum odour value. This happens, for example, when some perfumes are detected after being applied to clothes that are kept closed in a drawer or in a closet for a long time (although only to the less volatile and more tenacious compounds are detected).

Figure 8.5 shows the location in the perfumery ternary diagram, PTD, of the initial composition points of the perfume test mixture corresponding to points P1 to P4. The “evaporation paths” (lines starting from point P_i) are also represented, showing the evolution of the mixture during evaporation in two ways:

- the change of the composition in the liquid phase (position in PTD);
- which is the component that has the maximum odour value in the gas phase, OV_{MAX} (type of point in PTD, Δ- -o).

We can see also that if the starting point is P1, the "evaporation path" passes through P2, P3 and P4. This happens because the volatility of A, P_{V_A}, is 77 times P_{V_B} and more than 12800 times P_{V_C}. This big difference in the volatility of the top note is the reason why the "evaporation path" is vertical in the PTD.

8.3.3 Effect of middle and base notes

Figure 8.6 shows the effect of the variation of middle and base notes, B and C, respectively, for a constant value of the top note, A, in the evaporation of the test mixture. Figure 8.6 a-b-c-d correspond to the evaporation of test mixtures for which the starting composition are represented by points P5 through P9, respectively, as indicated in Figure 8.7. In all cases the initial molar fraction of A is constant and equal to x_{A0} =0.733, the molar fractions of B increases from points P5 through P9 and the molar fraction of C decreases from points P5 through P9 (see Table 8.2). These points belong to a horizontal line in the corresponding perfumery ternary diagram (PTD (see Figure 8.7).

In the first minutes of evaporation, initial test mixtures corresponding to points P5, P6 and P7 have an headspace with $OV_{MAX} = OV_A$ (Figure 8.6 a-b-c), while initial test mixtures corresponding to points P8 and P9 have an headspace with $OV_{MAX} = OV_C$ (Figure 8.6 d-e).This behaviour was predicted by the perfumery ternary diagram theory, as it can be seen in Figure 8.7.

When starting from the composition of point P5, $OV_{MAX} = OV_A$ during the first 55.2 minutes. After this time, $OV_{MAX} = OV_B$. Starting from point P6, $OV_{MAX} = OV_A$ during the first 70.8 minutes and after that, again $OV_{MAX} = OV_B$. After a long time, $OV_{MAX} = OV_C$ because the evaporation path follow the base line $\overline{BC}$ of the PTD (side BC of the triangle) until corner C (pure C), as can be seen in Figure 8.7. If starting from P7, then limonene is the component with the highest OV, $OV_{MAX} = OV_A$, during the first 58.8 minutes but after this time vanillin becomes the dominant odour, with $OV_{MAX} = OV_C$. In this last case, geraniol has never the maximum OV during evaporation of the perfume test mixture. Again, this effect was predicted in the corresponding PTD of Figure 8.7.

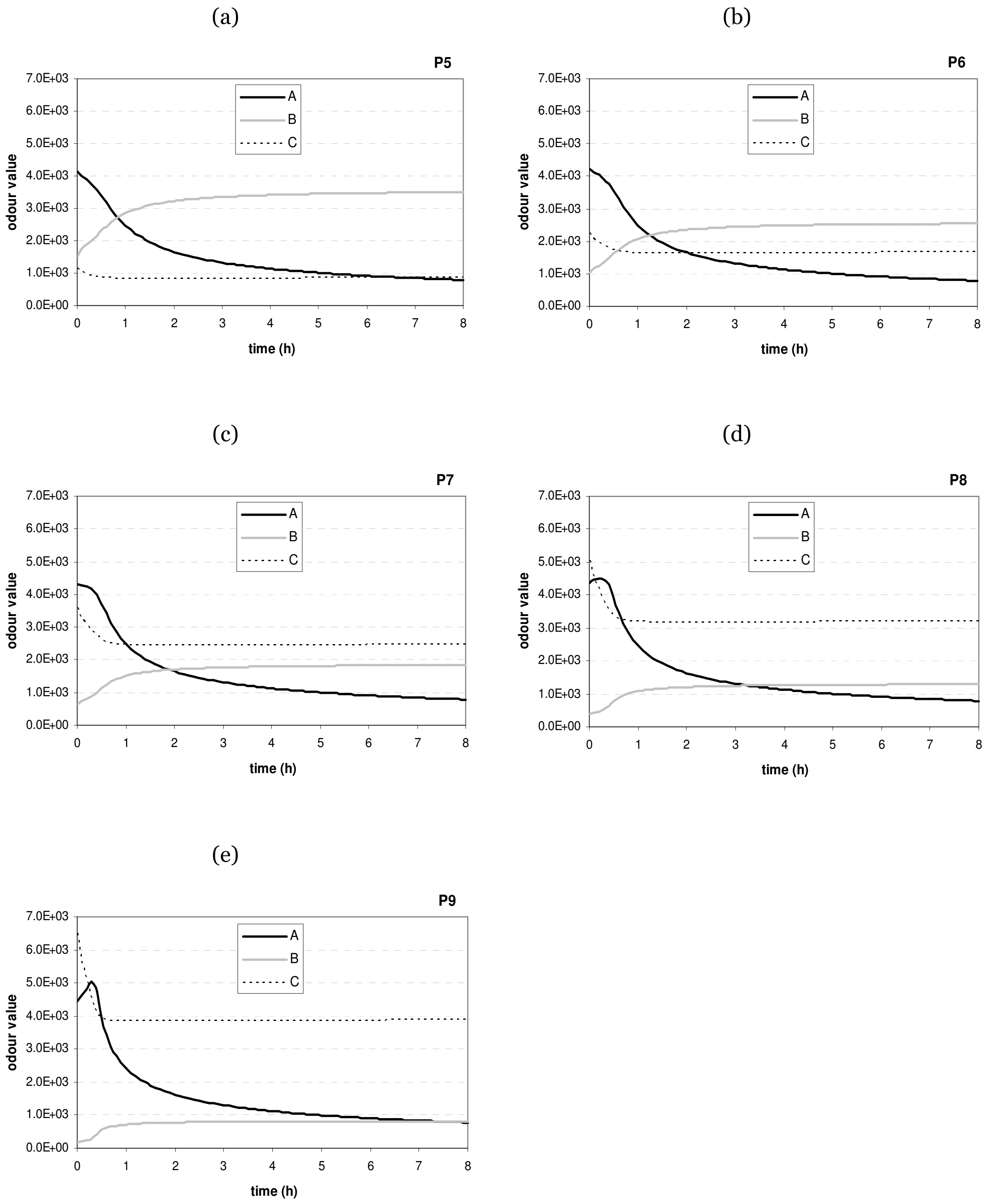


Figure 8.6 Variation of odour value as a function of time using an initial composition represented by points shown inTable 8.2, with limonene (A), geraniol (B) and vanillin (C): (a) P5; (b) P6; (c) P7; (d) P8; (e) P9.

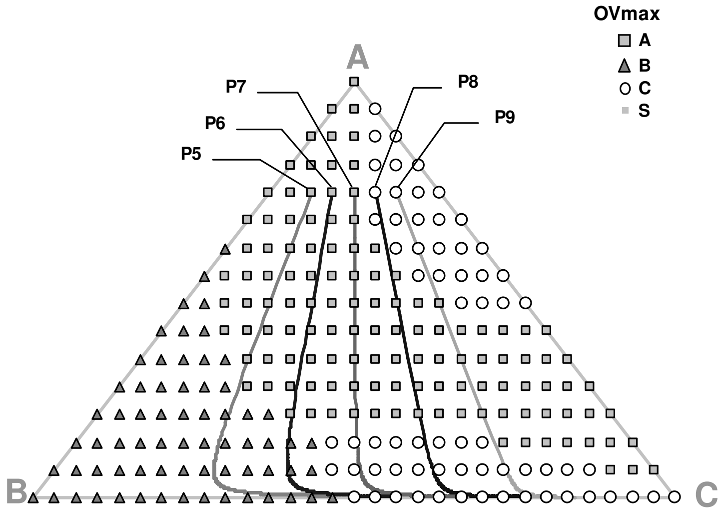
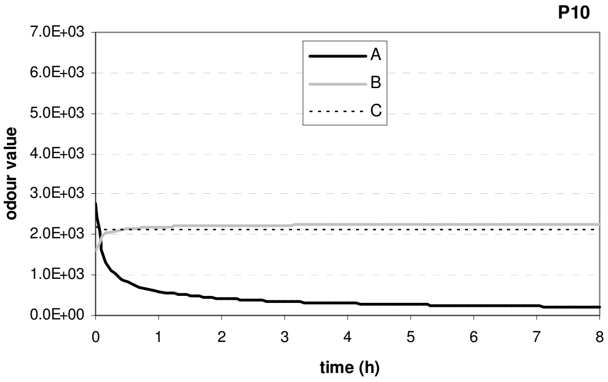


Figure 8.7 Evaporation paths represented in the perfumery ternary diagram of the test mixture, composed of limonene (A), geraniol (B) and vanillin (C), for the starting compositions P5, P6, P7, P8 and P9.

Figure 8.6 d-e are special cases. Although the molar fraction of A is high, when starting from the composition of points P8 and P9, it is vanillin that has the maximum OV, $OV_{MAX} = OV_C$. This occurs until 10.8 min for point P8 and 16.8 for point P9. After this time and during some time interval, $OV_{MAX} = OV_A$. These intervals of time are: from 10.8 to 33.6 min for point P8 and from 16.8 to 22.8 min for point P9. Finally, $OV_{MAX} = OV_C$ and in both cases there are no points where $OV_{MAX} = OV_B$.

Another special case is presented in Figure 8.8, where the composition of the initial test mixture is represented by point P10 (see Table 8.2, Figure 8.7). This evaporation path passes through four different phases: (i) 0<t<1.2min, $OV_{MAX} = OV_A$; (ii) 1.2<t<22.8min, $OV_{MAX} = OV_C$; (iii) 22.8min<t<69.0h, $OV_{MAX} = OV_B$; (iv) t> 69.0 h, $OV_{MAX} = OV_C$. This effect is well seen in the PTD of Figure 8.9.

(a)



(b)

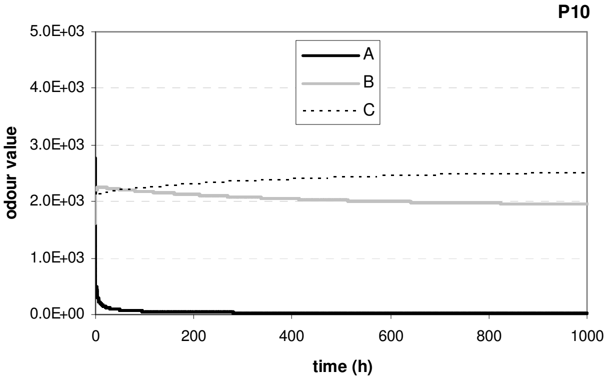


Figure 8.8 Variation of odour value as a function of time using an initial composition composed of limonene (A), geraniol (B) and vanillin (C), represented by point P10: (a) during 8 hours; (b) for a very long time (1000 hours).

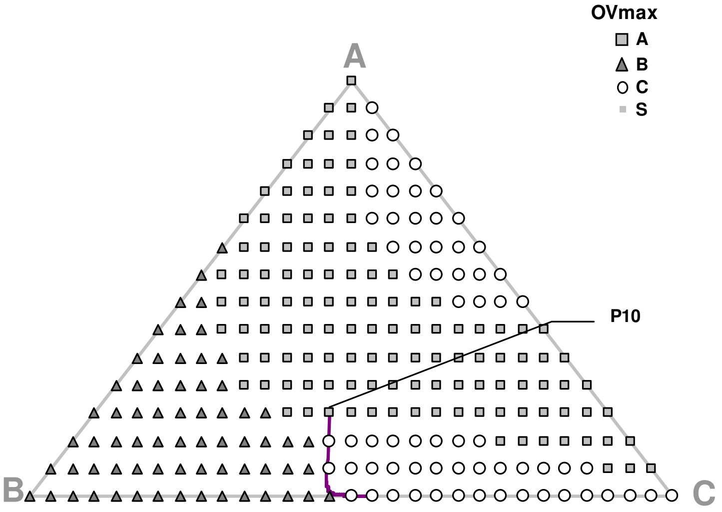


Figure 8.9 Evaporation path represented in the perfumery ternary diagram of the perfume test mixture, composed of limonene (A), geraniol (B) and vanillin (C), for the starting compositions P10.

8.3.4 Evaporation over distance

Figure 8.10 shows the concentration profiles for limonene (Figure 8.10-a), geraniol (Figure 8.10-b) and vanillin (Figure 8.10-c) in the gas phase, for a distance between $z = 0$ m and $z_{\max} = 2$ m, during 2 hours, obtained using Equation 8.1.

The more volatile component – limonene - evaporates very quickly, as it can be seen in the fast decrease of concentration at the interface, $z = 0$. Geraniol and vanillin concentration decrease more slowly, because of the very low saturated vapor pressure, at the system temperature (25°C).

Notice that the equilibrium at the gas-liquid interface and the remaining gas phase profiles are reached in a stagnant medium, where there is no convection.

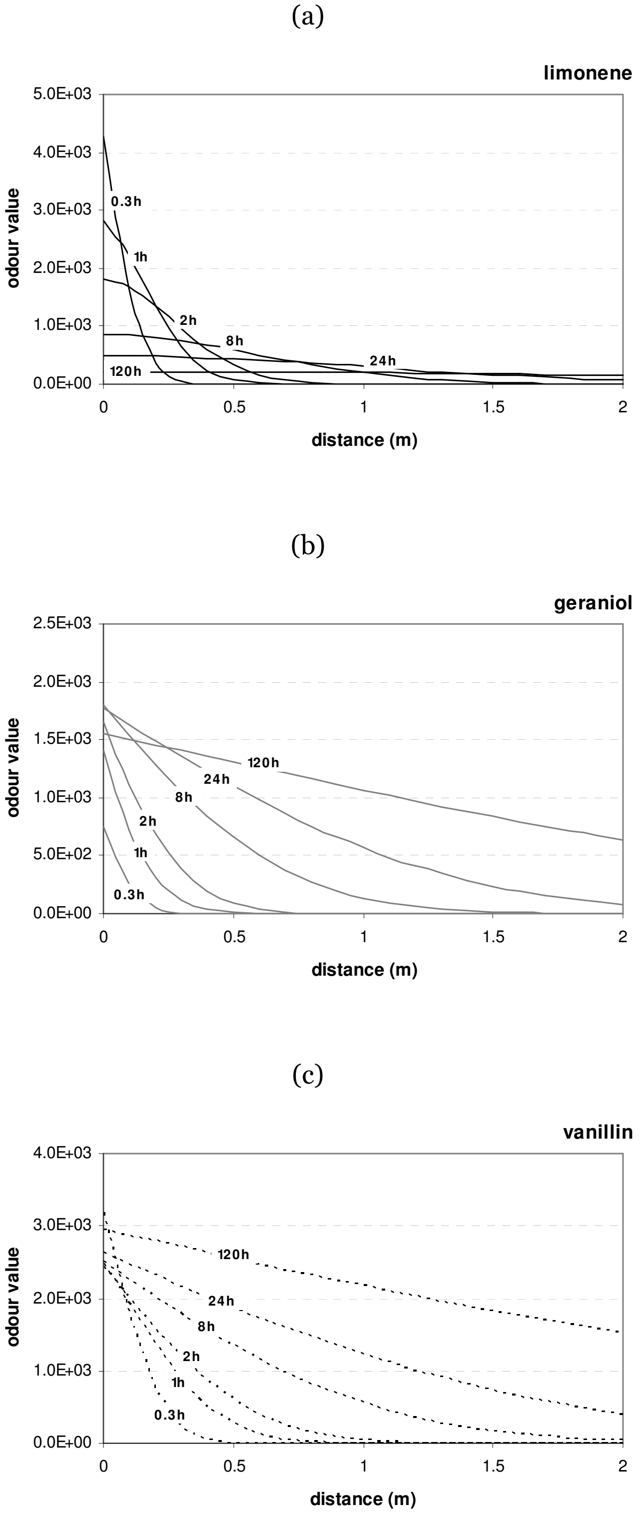


Figure 8.10 Odour value, OV, of each component in the gas phase, as a function of distance: (a) limonene, (b) geraniol and (c) vanillin.

8.3.5 Study of odour performance criteria

The odour value variation in time, at different distances, z_{nose} (see Figure 8.2) from the liquid mixture, was studied. There were considered the points: $z = 0$ m (gas-liquid interface) (Figure 8.11-a), $z = 0.3$ m (Figure 8.11-b) and $z = 1$ m (Figure 8.11-c). The composition of this liquid mixture is represented by composition point P1 (see Table 8.2).

These Figures can be used to illustrate the different aspects of odour performance, namely: impact, tenacity, diffusion and volume. These perfume performance parameters will be evaluated using Odour Values (OV), being OV a measure of odour intensity, and assuming that the component will have better performance if its intensity (or OV) is higher than the other components (Appell, 1969).

Once more, it must be taken into account that this study is done for a small and finite volume of a perfume mixture.Figure 8.11-a shows the case $z = 0$ m (point at the gas-liquid interface) that represents, for example, smelling directly a perfume bottle or a paper bottler. At this distance it is possible to evaluate impact (shortly after application) and tenacity (some time after application). The highest OV is that of limonene in the initial minutes, when compared to the OV of geraniol and vanillin. This means that limonene has a higher impact than the other two components. However, after some time - $t > 1.2h$, the highest OV is that of vanillin (and the OV of geraniol is similar) meaning that these two compounds – geraniol and vanillin – have higher tenacity than limonene. They prevail in the air more time, because they are more persistent.

In Figure 8.11-b we see that for a smelling distance $z = 0.3$ m the OV of vanillin is the highest one and the OV of limonene is still higher than that of geraniol until 8h after application. This means that vanillin has a higher diffusion that the other two compounds; in fact, Table 8.1 indicates that the diffusion coefficient of vanillin in air is almost twice that of geraniol and of limonene. In this Figure and in the next Figure 8.11-c, it can be seen that the component that can reach longer distances after a long time is vanillin. Its effectiveness is higher than the other two compounds, having this way a higher volume.

The classification of odour performance parameters is usually done qualitatively, based on words such as “soon” and “some” time after application, “near” and “at some” distance from the application point. In order to quantify these odour parameters, time and space variables must be specified.

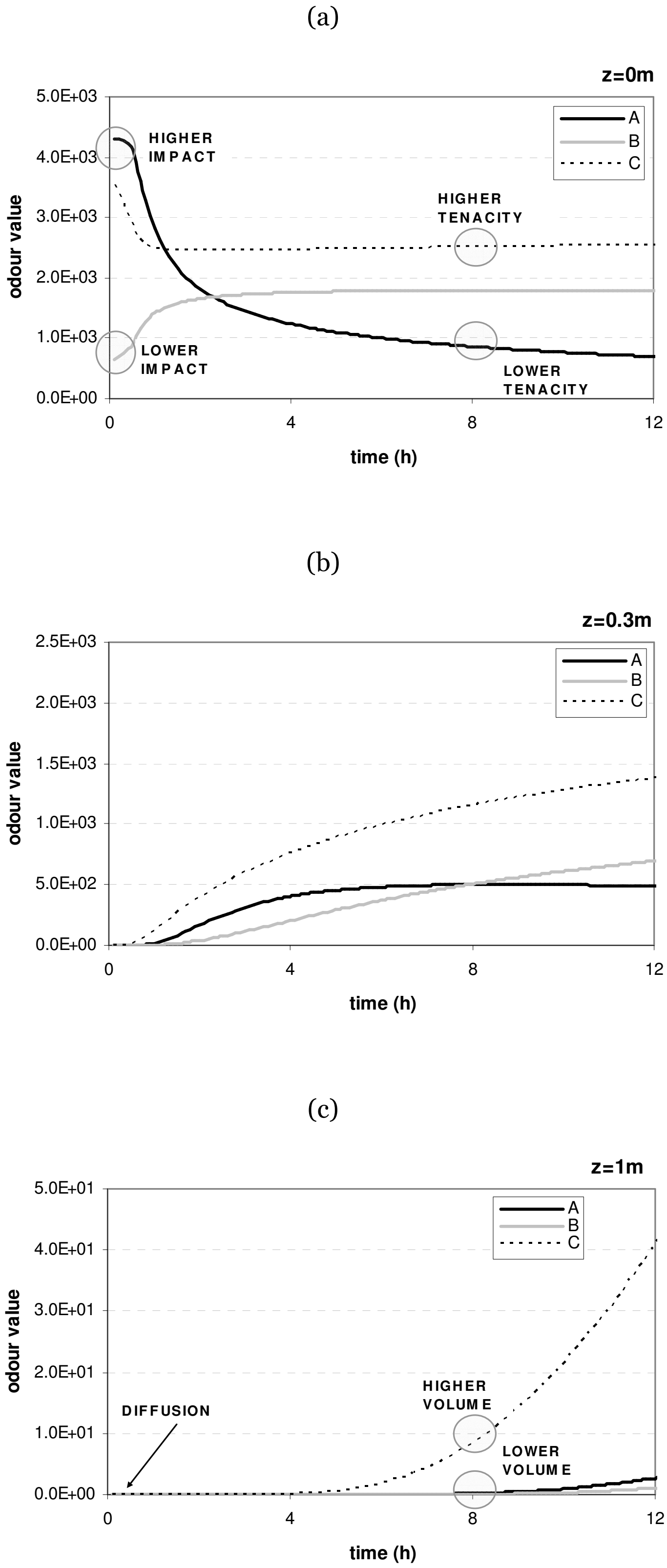


Figure 8.11 Variation of odour value as a function of time for different smelling distances, with limonene (A), geraniol (B) and vanillin (C).: (a) 0 m, (b) 0.3 m and(c) 1.0 m.

Let us assume that "soon" after application will be 1 min, "near" application point is 0.01 m, "some time" after application will be 8h and "some distance" will be 1 m. This way, the odour parameters will be evaluated the following way: impact (t=1min, z=0.01m), diffusion (t=1min, z=1m), tenacity (t=8h, z=0.01m) and volume (t=8h, z=1m).

As odour is always considered in relative terms by comparison with other odours, OV values should also be considered relatively to some reference value. New variables can then be defined to quantify the odour parameters, as follows:

- Impact level, IL_i, defined as the ratio of OV_i (t=1min, z=0.01m) and OV_{ref};
- Diffusion level, DL_i, defined as the ratio of OV_i (t=1min, z=1m) and OV_{ref};
- Tenacity level, TL_i, defined as the ratio of OV_i (t=8h, z=0.01m) and OV_{ref};
- Volume level, VL_i, defined as the ratio of OV_i (t=8h, z=1m) and OV_{ref};

For example, if limonene is chosen as a reference compound, OV_{ref} can be $OV_{A\max}$ that corresponds to OV_A value when $x_A = 1$. In this case, $OV_{ref} = 4.6\times10^3$. The odour performance parameters quantified this way are shown in Table 8.3, for the same composition point P1. It can be seen that it is in agreement with the previous qualitative considerations, namely: vanillin is the component with highest diffusion, tenacity and volume, while limonene is the one with the highest impact. Moreover, quantitative comparison can also be made: limonene has an impact level 1.2 times higher than vanillin, but vanillin has a tenacity level and volume level 3 times higher than limonene. Comparing vanillin with geraniol, vanillin has an impact level 6 times higher, a diffusion level 4.6 times higher, a tenacity level 1.4 times higher and a volume level 4.1 times higher. Comparing limonene and geraniol, limonene has an impact level 7 times higher, a diffusion level 4 times higher and a volume level 1.5 times higher than geraniol, while the tenacity level of geraniol is twice of that of limonene.

Table 8.3 Odour performance parameters and component with maximum performance, using OV of pure limonene as reference.

	Component	IL_i	DL_i	TL_i	VL_i
A	limonene	9.4×10^{-01}	3.6×10^{-14}	1.9×10^{-01}	4.2×10^{-02}
B	geraniol	1.3×10^{-01}	9.5×10^{-15}	3.9×10^{-01}	2.9×10^{-02}
C	vanillin	8.1×10^{-01}	4.3×10^{-14}	5.4×10^{-01}	1.2×10^{-01}
	MAXIMUM	A	C	C	C

8.4 Conclusions

A methodology to predict the evaporation of binary and ternary perfume mixtures in a solvent base was developed. The method is based on the concept of Perfumery Ternary Diagrams (PTD) and on a diffusion model for predicting the evolution of odour values (OV) in time and space.

PTD allows us to pick an initial perfume composition and predict its evaporation path that is showing: (i) the changes in the composition the liquid phase and (ii) changes in OV of the headspace.

The simple diffusion model developed predicts the evaporation of the small perfume mixture as a function of time and distance from the application point of the liquid sample. The results obtained were used to evaluate the performance criteria of the ternary perfume mixture in the headspace by the following parameters: impact, tenacity, diffusion and volume. In this way, the performance evaluation is more objective than the normal practice in perfumery, because it was based on the comparison of odour values, OV, quantifiable, instead of being established in an intuitive or experimental way, and so very subjective.

A time and space reference, as well as an odour reference, were arbitrarily chosen in order to quantify these odour performance criteria. There were defined four new parameters: impact level, diffusion level, tenacity level and volume level. This new variables are useful for comparing in quantitative terms (more objective) but note that they are only meaningful in relative terms, with their values depending on the odour reference chosen. For the specified time and space reference, it was found that limonene is the compound with higher impact and vanillin is the one with higher diffusion, tenacity and volume.

8.5 References

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CHAPTER 9 CONCLUSIONS AND FUTURE WORK

In this chapter, a summary of the conclusions of this thesis is given, and suggestions for future research are given.

9.1 Conclusions

This thesis addresses several aspects related with the perfumery industry, namely:

(i) the screening and characterization of two natural perfume materials with economic potential to be explored as a natural national resource, namely rose geranium (*Pelargonium sp*) and rockrose (*Cistus ladanifer*);

(ii) the extraction of naturals using supercritical fluid extraction as a clean technology to produce naturals with improved organoleptic qualities;

(iii) the fractionation by preparative liquid chromatography of essential oils to produce terpeneless oils due to their high-added value for flavours & fragrances;

(iv) the formulation of perfumes with scientific basis, using a new strategy based on Perfumery Ternary Diagrams;

(v) the prediction of perfume behaviour during evaporation in time and space using a diffusion model, in order to evaluate objectively the performance using quantitative parameters.

The main conclusions of this thesis are reviewed next.

The Portuguese-grown geranium essential oil (*Pelargonium sp*) herein obtained had good olfactory quality, resembling the best quality geranium oil of Bourbon type but presenting characteristics different from those found in the literature. The cistus oil obtained from rockrose (*Cistus ladanifer*) was richer in amber-like components, such as ambrox with ambergris scent, and had a low content of monoterpenes when compared to the usual values found in the literature and in commercial cistus oils.

A small-scale pilot plant for supercritical fluid extraction (SFE) was designed and constructed during this work. The design of this SFE equipment, named SCRITICAL resulted in a compact, versatile and portable equipment, having the following advantages: requires less laboratory space to accommodate; minimises dead volumes; has an easy monitoring; is economical, and most important it can be operated at higher flow rates and with different configurations (for example, using different extractor and separator volumes, add extra regulation valves, extraction and separation vessels), options which are not possible or difficult to implement in available commercial laboratory equipments.

The clean technology of CO2 SFE was employed for the extraction of rose geranium plant (*Pelargonium sp*), characterized within this work. The supercritical extracts showed a considerable high quality in terms of odour and colour, with very fresh natural floral-fruity character and a pale yellow colour, most suitable for the use in perfumery. The optimum SFE conditions were found to be: extraction time of 15-30 min, temperature of 40 °C, pressure of 90-100 bar, cut fresh plant and dynamic mode.

The fractionation of essential oils by preparative liquid chromatography was applied to the case of deterpenation of citrus oils using silica as adsorbent. The selection of mobile phase that will provide effective separation and better productivity and economy is a critical step in defining a chromatographic separation. In this work, a strategy to optimise the separation and select the best mobile phase was developed using a chromatographic model. There were found two mobile-phases that accomplish the separation of the two components: heptane/ethyl acetate 85:15 and hexane/ethanol 85:15. In optimised conditions, heptane/ethyl acetate 85:15 provided the best productivity and the least solvent consumption, though in elution mode it was hexane/ethanol 85:15 that was better since the retention times were smaller.

A new methodology based on the concept of Perfumery Ternary Diagrams (PTD) was used to predict the behavior perfume binary and ternary mixtures. PTD allowed the calculation of the headspace odour value, OV, for any combination of these materials in the corresponding liquid solution, and based on the relation OV-intensity it is possible to infer about how it will smell without making too many experimental trial and error combinations. Therefore, PTD can be used as compounding tool for making the perfume mixtures.

The Perfumery Ternary Diagram (PTD) methodology was validated by experimental measurement of headspace concentrations, vapour pressures and odour threshold values at 25 °C. The Perfumery Ternary Diagrams (PTD) profile obtained for the perfume test mixture composed of limonene, geraniol and vanillin, changed significantly from the one using the data found in the literature, mainly due to the differences verified between the experimental values and the literature data for odour threshold values. Comparing the odour values (OV) predicted by PTD model and the ones obtained based on experimental data, it was concluded that the OV for the non-polar component (limonene) and medium-polar component (geraniol) was reasonably well predicted, while the OV for the polar components (vanillin and ethanol) was poorly estimated, showing that the UNIFAC model should be improved by introducing new structural groups that take into account the complexity of the fragrance molecules, which include cyclic components with many functional groups, or use other models such as Modified UNIFAC or NRTL. Nevertheless, the methodology has proven to be useful in predicting OV values of perfume mixtures, giving some insight on the behaviour of the components in solution, and how that affects the headspace composition and its odour.

The evaporation of the small perfume mixture after application, as a function of time and distance, was predicted by a diffusion model. The results obtained were used to evaluate the performance criteria of the ternary perfume mixture in the headspace based on calculated odour values: impact, tenacity, diffusion and volume. There were defined four new parameters for comparing performance in quantitative terms: impact level, diffusion level, tenacity level and volume level. This way it was possible to assess objectively and quantitatively the performance, which is normally established in an intuitive or experimental way, often using qualitative terms thus considerably subjective.

9.2 Future Work

Many other aromatic plants may be explored for flavour & fragrance applications, with high potential to be cultivated in Portugal. Our favourable climate and soil may be well used to produce naturals with high demand, as is the case of the studied rose geranium oil. An economical analysis is necessary though, in order to choose the best production process, namely supercritical fluid technology, distillation, extraction with organic solvents or fractionation using preparative chromatography.

The SFE equipment herein constructed, SCRITICAL, may be improved by setting automatic control, use a better high-pressure pump (diaphragm or membrane pump), pressurize the system before starting extraction in order to speed up stabilization time, introduce more depressurization steps to prevent more efficiently freezing due to Joule-Thomson effect and to fractionate the extract, and eventually be adapted to perform supercritical fluid chromatography for the fractionation of essential oils.

Considering the perfume composition, the Perfumery Ternary Diagram (PTD) should use other thermodynamic models so that it can predict more accurately the behaviour of the fragrant substances. The application of PTD methodology should also be extended to more complex systems.

The evaporation model should also be validated experimentally, including the measurement of the real values for odour performance parameters: impact, tenacity, diffusion and volume. Moreover, the evaporation from different substracts and media should be studied, such as from skin, hair and textiles, as well as considering the influence of application and diffusion method (spray, thin film, heating).

There is a great variety of kinds of perfumes, which are classified into four main odour families, namely oriental, floral, chypre and fougère, which in turn are classified in sub-families or facets such as fresh, transparent, maritime/ozonic, green, musky, aldehydic, powdery, sweet, woody. These odour families differ in their dominant fragrant notes and the classification is made by the perfumers, having a considerable level of subjectivity because it depends on their experience and sense of smell. Consequently, there are many classifications of perfumes, which often are not consistent. For example, CK One is classified as Chypre Fresh by H&R Genealogy, Fresh Natural by Dragoco Octagon and Hesperedic by Cinquième Sens; another example is 24, Faubourg that is Floral according to H&R Genealogy, Chypre to Dragoco Octagon and Floral-Oriental to Cinquième Sens. Considering this, it would be very useful to find a systematic classification into odour families, based on their composition (quantitative) instead of being based only on the evaluation by smell. The PTD methodology herein developed, together with the GC/MS analysis of perfumes could be used to find some graphical representation based on odour values that would allow inferring about the odour family of any perfume. Then it would also be possible to do the reverse path, which would be to choose first an odour family, and then formulate the composition having the aimed dominant note.

An area in F&F that was not covered in this thesis was the synthesis of fragrant molecules. The know-how of LSRE laboratory in separation and reaction processes could be extended to the production of natural-identical and new artificial raw materials for perfumery.

A. CALCULATION OF THE ACTIVITY COEFFICIENTS USING THE UNIFAC METHOD

The values of activity coefficients, γ_i, were calculated using the UNIFAC method, which is based on molecular group contribution for multi-component mixtures (Fredenslund *et al.*, 1975; Reid *et al.*, 1987). This method takes into account the contribution due to differences in molecular size and shape – combinatorial part (C) - and the contribution due to molecular interactions – residual part (R), in the following way:

$$\ln \gamma_i = \ln \gamma_i{}^C + \ln \gamma_i{}^R \tag{A.1}$$

A.1 Determination of the combinatorial part

The combinatorial part is calculated using the following equation:

$$\ln \gamma_i{}^C = \ln \frac{\phi_i}{x_i} + 5 q_i \ln \frac{\theta_i}{\phi_i} + l_i - \frac{\phi_i}{x_i} \sum_j x_j l_j \tag{A.2}$$

where

$$l_i = 5(r_i - q_i) - (r_i - 1) \tag{A.3}$$

$$\theta_i = \frac{q_i x_i}{\sum_j q_j x_j} \tag{A.4}$$

$$\phi_i = \frac{r_i x_i}{\sum_j r_j x_j} \tag{A.5}$$

Parameters r_i and q_i are calculated for each molecule as the sum of the group volume and area parameters, R_j and Q_j, as follows:

$$r_i = \sum_j \nu_j^{(i)} R_j \tag{A.6}$$

$$q_i = \sum_j \nu_j^{(i)} Q_j \tag{A.7}$$

where $\nu_j^{(i)}$ is the number of groups of type j in molecule i. Group parameters are obtained by the van der Waals group volume and surface areas, shown in Table A.1 and Table A.2.

A.2 Determination of the residual part

The residual part is calculated using the following equation:

$$\ln \gamma_i{}^R = \sum_{\substack{k \\ all\,groups}} \nu_k^{(i)} \left(\ln \Gamma_k - \ln \Gamma_k^{(i)} \right) \tag{A.8}$$

where Γ_k is the group residual activity coefficient and $\Gamma_k^{(i)}$ is the residual activity coefficient of group k in a reference solution containing only molecules of type i.

$$\ln \Gamma_k = Q_k \left[1 - \ln \left(\sum_m \theta_m \psi_{mk} \right) - \sum_m \frac{\theta_m \psi_{km}}{\theta_n \psi_{nm}} \right] \tag{A.9}$$

Equation (A.9) also holds for $\ln\Gamma_k^{(i)}$. In this equation, θ_m is the area fraction of group m, and the sum is over all different groups, as:

$$\theta_m = \frac{Q_m X_m}{\sum_n Q_n X_n} \tag{A.10}$$

where X_m is the mole fraction of group m in the mixture. The group interaction parameter ψ_{mn} is given by:

$$\psi_{mn} = \exp\left(-\frac{a_{mn}}{RT}\right) \tag{A.11}$$

Parameters a_{mn} and a_{nm} has units of Kelvin and their values are found in the literature (Reid *et. al*, 1987).

Table A.1 UNIFAC groups and subgroups for the components in the perfume test mixture: limonene, geraniol, vanillin and ethanol (in Reid et. al, 1987).

Molecule (i)	Subgroup (j)	Subgroup number	Group number	$\nu_j^{(i)}$
limonene	CH_3	1	1	2
	CH_2	2	1	3
	CH	3	1	1
	$CH = C$	8	2	1
	$CH_2 = C$	7	2	1
geraniol	CH_3	1	1	4
	CH_2	2	1	2
	$CH = C$	8	2	2
	OH	15	5	1
vanillin	ACH	10	3	3
	AC	11	3	2
	$ACOH$	18	8	1
	CHO	21	10	1
	CH_3O	25	13	1
ethanol	CH_3	1	1	1
	CH_2	2	1	1
	OH	15	5	1

Table A.2 UNIFAC groups and subgroups for the aroma chemicals used in systems S1-S4, in Chapter 6 (in Reid et. al, 1987).

Molecule (i)	Subgroup (j)	Subgroup number [1]	Group number[1]	$\nu_j^{(i)}$
benzaldehyde	ACH	10	3	5
	AC	11	3	1
	CHO	21	10	1
phenyletanol	ACH	10	3	5
	$ACCH_2$	13	4	1
	CH_2	2	1	1
	OH	15	5	1
eugenol	$ACOH$	18	8	1
	ACH	10	3	3
	$ACCH_2$	13	4	1
	$CH = CH_2$	5	2	1
	CH_3O	25	13	1
α-pinene	CH_3	1	1	3
	CH_2	2	1	2
	CH	3	1	2
	$CH = C$	8	2	1
	C	4	1	1
linalool	CH_3	1	1	3
	CH_2	2	1	2
	C	4	1	1
	$CH = C$	8	2	1
	$CH_2 = CH$	5	2	1
	OH	15	5	1
tonalide	CH_3	1	1	6
	CH	3	1	1
	C	4	1	2
	$CH = C$	8	2	2
	$C = C$	9	2	1
	$CH_3 = CO$	19	9	1

[1] Reid, R.C.; Prausnitz, J.M.; Poling, B.E.; *The properties of Gases and Liquids*. McGraw-Hill, NY, **1987**.

A.3 References

Fredenslund, A., Jones, R. L. and Prausnitz, J. M., *AIChE J.*, **21**, 1086 (1975).

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B. CALCULATION OF THE DIFFUSION COEFFICIENTS USING THE FULLER ET AL. METHOD

In this work, as the components are very diluted in air, the diffusion coefficient of each component i in air, j, were estimated using the correlation of Fuller et. al. [22] for a given pressure and temperature, using the following equation:

$$D'_{ij} = CT^{1.75} \frac{\sqrt{\frac{M_i + M_j}{M_i M_j}}}{P\left\{\sqrt[3]{V_i} + \sqrt[3]{V_j}\right\}^2} \qquad [A5]$$

with T in Kelvin (K), P in Pascal (Pa), M_i and M_j in grams per mole (g/mol) and $C = 1.013 \times 10^{-2}$, and D'_{ij} in m²/s. The terms V_i and V_j are molecular diffusion volumes and are calculated by summing the atomic contribution in Table B.1 (only the values used in this work). For example, the molecular formula of ethanol is C_2H_6O. Then, $V_{ethanol} = 2 * V_C + 6 * V_H + 1 * V_O$.

Table B.1 Atomic contributions for the calculation of molecular diffusion volumes.

m	**Atomic and molecular diffusion volumes increments, V_m**
C	15.9
H	2.31
O	6.11
Aromatic Ring	-18.3
Air	19.7

C. CALCULATION OF ODOUR THRESHOLD VALUES

This appendix gives an explanation for the procedure used during the determination of the odour threshold values based on the data obtained using an olfactometer.

The data file obtained comprises a matrix of all the answers of the panellists, a table with the dilution factor corresponding to the first positive answer, odour threshold concentration (Z_{50}) and other statistical parameters. The calculations necessary to determine the odour threshold concentrations will be explained by an example.

C.1 Example of test measurement

The matrix of the answers given by all the panellists is presented in Table C.1. The first column represents the identification of the panellists (P1 to P4), while the first row refers to the kind of air smelled by the panellist: R – reference or neutral air, B – blank (neutral air) and odorant air at the indicated dilution ratio. The cross,×, means that the panellist pressed the button at that dilution step as a positive answer.

Table C.1 Matrix of the answers given by the panellists for the 3 sequences of a test measurement.

sequence 1

	R	B	R	B	R	160	R	80	R	40	R	20	R	10	R	5
P1										x		x				
P2								x		x		x				
P3										x		x				
P4										x		x				

sequence 2

	R	B	R	B	R	160	R	80	R	B	R	40	R	20	R	10
P1												x		x		x
P2								x				x		x		x
P3														x		x
P4												x		x		x

sequence 3

	R	B	R	B	R	160	R	80	R	40	R	20	R	B	R	10
P1										x		x				
P2										x		x				
P3										x		x				
P4										x		x				

From this matrix of answers the table of results of the panellists (Table C.2) is obtained, containing the dilution factor corresponding to the first positive answer and that will be the basis for the odour threshold determination.

The calculation of odour threshold is based on the decimal logarithm of the dilution factor because empirically it was found that the distribution of the logarithms of the matrix of results is normal, in a first approximation (VDI 3881).

Table C.2 Results of the panelists.

Sequence	**Start step**	**Pre-dilution**	P1	P2	P3	P4
1	160	1	40	80	40	40
2	160	1	40	80	20	40
3	160	1	40	40	40	40

The dilution factor at threshold (Z_{thr}) per sequence and per panellist is given by the geometric mean of the dilution factors corresponding to the first positive answer (Z_N) at dilution step N and the last negative answer (Z_{N-1}) at the previous dilution factor step ($N-1$):

$$Z_{thr} = \sqrt{Z_N \times Z_{N-1}} \qquad [C.1]$$

The threshold dilution factors per sequence and for each panellist are shown in Table C.3.

Table C.3 Threshold dilution factors for all panellists.

Sequence	P1	P2	P3	P4
1	57	113	57	57
2	57	113	28	57
3	57	57	57	57

The arithmetic mean (M)and standard deviation (s) of the logarithms of all threshold dilution factors are calculated by the equations:

$$M = \frac{\sum_{k=1}^{N} \sum_{i=1}^{3} \log_{10}(Z_{thr\,k,i})}{L} \qquad [C.2]$$

$$s = \sqrt{\frac{\sum_{k=1}^{K} \sum_{i=1}^{3} \left(\log_{10}(Z_{thr\,k,i}) - M\right)^2}{L-1}} \qquad [C.3]$$

where $Z_{thr\,k,i}$ is the threshold dilution factor for panellist k and at measuring sequence i, K is the total number of panellists and L is the total number of answers (equal to 3 × K).

Finally the threshold dilution factor for this measurement is calculated as:

$$Z_{50} = 10^{M} \qquad [C.4]$$

and the odour threshold concentration is given by:

$$C_{thr} = \frac{C_0}{Z_{50}} \qquad [C.5]$$

where C_0 is the concentration of the odour sample.

The reaction limit and the determination limit are given by:

$$Z_{16} = 10^{M+s}$$

$$Z_{84} = 10^{M-s}$$

The resolution is expressed as:

$$\lambda = \frac{1}{2}\log_{10}\left(\frac{Z_{16}}{Z_{84}}\right)$$

For this test example, the results are:

$Z_{50} = 60$

$Z_{16} = 86$

$Z_{84} = 42$

$\lambda = 0.155$

Considering that the fragrant gas sample was prepared with a concentration of 120 mg/m^3, then the threshold concentration is:

$$C_{th} = \frac{120}{Z_{50}} = \frac{120}{60} = 2 \ mg/m^3$$