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Extraction of volatile aroma compounds of olive oil for odour standards to be used in the training and monitoring of sensory analysis panels: a preliminary study

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Abstract

The unique and delicate flavour of olive oil is attributed to a number of volatile components. These volatile flavour compounds are formed in the olive fruit through an enzymatic process. Olive cultivar, origin, maturity stage of fruit, storage conditions of fruit, and olive fruit processing influence the flavour components of olive oil and therefore its taste and aroma.

Sensory analysis is an essential part of evaluating olive oil quality and legal governmental requirement for determining the quality of olive oil, realised through panel tests, based on the standards of the International Olive Council (IOC) and primarily on the Regulation (EC) 640/2008 of the European Commission.

In today's competitive marketplace, the ability to describe the odour of products in objective terms and to check their conformance with pre-defined quality standards is increasingly critical to the commercial success. Often the difficulty of this type of training resides in the impossibility of obtaining reference standards that can be used by different panels, allowing to compare their performance.

The aim of this work was to contribute through extraction methods to the preparation of odour standards corresponding to the defects and characteristic scents, prepared out of 34 typical and atypical olive oil samples.

For this purpose, in a first step the samples were analysed by HS-SPME in combination with GC-MS. In average 172 volatile compounds were identified, of which in average 51 per sample could be identified through literature research being of sensory relevance and establish an association to their respective sensory attributes and the olive oils volatile profile.

In a second step, Supercritical fluid extraction (SFE) and distillation for the extraction of the volatile compounds were tested. The results obtained showed amongst others:

- In SFE more drastic extraction conditions (higher temperature and pressure) led to the obtaining of chromatograms with peaks that showed higher areas;
- Of the collecting solvents tested, 20% ethanol proved to be the most efficient, however the extracts did not smell similar to an olive oil sample and their sensory evaluation was impaired by the smell of ethanol.
- The profile of the volatile compounds in the extracts was characterized by the presence of compounds common for oxidation of the olive oil sample.
- Distillation compared to SFE resulted in higher percentual peak areas and a higher number of extracted compounds of interest. The higher percentage of aldehydes observed in the extracts probably resulted from the oxidative alteration of the sample, rather than a higher extraction efficiency.

Considering the poor extraction results, it was possible to build two hypotheses:

1. The extraction step was not as successful as intended;
2. The conditions used for the trapping of the compounds were not the most appropriate or effective enough for trapping the target analytes. In fact, the success of

an extraction method depends not only on the extraction step itself, but also on the matrix considered and the analyte trapping system.

Keywords: Volatile compounds, olive oil, Supercritical fluid extraction SFE, Distillation, odour standards

Resumo em português

Até 2030, a produção de azeite da UE deverá aumentar 1,3% ao ano, impulsionado principalmente por novas plantações e melhorias nas práticas agronómicas. A nível dos Estados-Membros, estes fatores incluem, por exemplo, investimentos em irrigação em Espanha e Portugal, juntamente com a modernização da indústria de moagem em Portugal. Na Península Ibérica prevê-se um aumento acentuado da produção em cerca de 2% ao ano, em comparação com a média em 2015-2017. Esta produção adicional pretende dar resposta à crescente procura por este tipo de produto, tanto a nível mundial, como na UE e consolidará a posição da UE como maior produtor e exportador mundial de azeite.

A importância económica do setor nacional do azeite tem boas perspectivas para o futuro, embora se preveja um ritmo de crescimento mais lento do que na última década. Este crescimento estará relacionado com o reconhecimento da qualidade do azeite nacional, bem como o dinamismo dos principais grupos nacionais de produtores e distribuidores de azeite, que contribuirão certamente para um aumento das exportações e, consequentemente, para um crescente peso económico do setor nacional de azeite.

O aumento da concorrência entre os produtores obriga a uma constante melhoria da qualidade do produto, assumindo uma grande importância para obter uma vantagem competitiva na internacionalização dos mercados.

A identificação de diferentes atributos da qualidade do azeite deve constituir um claro incentivo para as empresas altamente competitivas e orientadas para o mercado interno e externo, a fim de satisfazer as necessidades de consumidores preocupados com a qualidade nas suas variadas vertentes, incluindo a qualidade sensorial. Na realidade, o azeite como gordura é muito apreciado pelos seus benefícios nutricionais, mas o azeite de alta qualidade, obtido a partir de frutas frescas e saudáveis também é apreciado pelo seu flavour delicado e único que é atribuído a vários componentes voláteis.

Estes compostos voláteis do flavour são sintetizados na azeitona. A cultivar de azeitona, a origem, o estado de maturação da fruta e as condições de armazenamento e processamento da fruta influenciam as características sensoriais deste tipo de produto.

A análise sensorial é uma parte essencial da avaliação da qualidade do azeite e complementa as análises químicas, que são requisitos legais para atribuir a classificação das amostras como azeite extra-virgem (EVOO), azeite virgem (VOO) ou azeite lampante em conformidade com o Regulamento (CE) 640/2008 da Comissão Europeia, sendo a avaliação feita por meio de testes com painéis sensoriais, com base nas determinações do Conselho Oleícola Internacional (IOC) e principalmente no Regulamento (CE) 640/2008 da Comissão Europeia.

Os painéis sensoriais são uma ferramenta essencial no controlo e desenvolvimento de alimentos e bebidas de alta qualidade. Num mercado competitivo, a caracterização objetiva

das características organolépticas dos produtos, que verifica a sua conformidade com padrões de qualidade predefinidos é cada vez mais crítico para o sucesso comercial. O maior problema reside na dificuldade em treinar os provadores dada a quase inexistência de padrões de referência que possam ser utilizados por diferentes painéis, permitindo comparar seu desempenho.

Como os materiais de referência actualmente usados são azeites virgens “naturais”, seleccionados por serem representativos de um único defeito sensorial, estes podem passar a ser levemente diferentes ano a ano em propriedades sensoriais e intensidade do defeito. Em contraste a isso, uma reprodutibilidade perfeita de cada defeito e odor característico seria extremamente útil para igualizar todos os painéis.

Para além de ajudar na classificação dos azeites, para a indústria do azeite os padrões de referência de odor poderiam ser usados para:

- a triagem e selecção de novos provadores;
- medir e melhorar o desempenho individual de membros do painel e de trainees;
- medir e melhorar o desempenho dos painéis sensoriais;
- medir e melhorar a eficácia dos programas de treinamento;
- ajudar a entender e definir a qualidade sensorial dos produtos;
- ou ajudar a identificar os diferentes descritores sensoriais dos produtos.

O objetivo deste trabalho foi contribuir através da utilização de diferentes métodos de extração, para a preparação de padrões de odor correspondentes aos defeitos e aromas característicos de azeites, preparados a partir de amostras típicas e atípicas.

Para este fim 34 amostras de azeite, das quais 13 com e 21 sem defeitos sensoriais, de diferentes regiões de Portugal foram analisadas por HS-SPME em combinação com Cromatografia Gasosa acoplada à Espectrometria de Massa (GC-MS). Os compostos voláteis de relevância sensorial presentes nas amostras foram identificados por recurso à biblioteca de espectros e índice de Kovats, bem como dados da literatura consultada.

A metodologia usada, HS-SPME-GC-MS permitiu identificar cerca de 172 compostos voláteis, em média, 51 por amostra puderam ser identificadas através de pesquisas bibliográficas de serem de relevância sensorial e assim estabelecer uma associação com seus respectivos atributos sensoriais e o perfil volátil do azeite.

O tratamento dos resultados para as diferentes amostras, por análise multivariada por componentes principais (ACP), permitiram a diferenciação das amostras com defeito sensorial das que não apresentavam defeito sensorial. As amostras defeituosas foram fortemente caracterizadas por teores mais elevados em compostos relacionados com o defeito a ranço, resultantes da oxidação do azeite virgem.

Numa segunda etapa do trabalho, foram testadas metodologias de extração dos compostos voláteis como a extração com fluido supercrítico (EFS) e a destilação. Os resultados obtidos mostraram que:

- Na EFS as condições de extração mais drásticas (temperatura e pressão mais altas) levaram à obtenção de cromatogramas que apresentavam picos com áreas superiores;
- Dos solventes coletores testados, a solução de etanol a 20% mostrou ser a mais eficiente, no entanto os extratos não apresentavam o odor semelhante a uma amostra de azeite e a sua avaliação sensorial era prejudicada pelo cheiro do etanol.
- Os perfis dos compostos voláteis nos extratos foram caracterizados pela presença de compostos comuns à oxidação da amostra de azeite.
- A destilação em comparação com a EFS resultou em áreas de pico percentuais mais altas e um número maior de compostos de interesse extraídos. A maior percentagem de aldeídos observada nos extratos provavelmente decorreu da alteração oxidativa da amostra, em vez de uma maior eficiência de extração.

Tendo em conta os resultados obtidos em que os extratos obtidos a partir dos azeites testados foram mais caracterizados por compostos comuns à oxidação, foi possível construir duas hipóteses:

1. A extração não ocorreu com a eficiência que se pretendia;
2. As condições usadas para o aprisionamento dos compostos não eram as mais adequadas. De facto o sucesso de um método de extração não depende apenas da etapa de extração, mas também da matriz considerada e do sistema de captura dos analitos.

Em conclusão vale dizer que a composição volátil e a relação entre os compostos voláteis e sua contribuição sensorial para o aroma geral do azeite na literatura parecem ser bem explorados. Apesar dos desenvolvimentos substanciais das técnicas de extração e separação, o isolamento de compostos naturais de matérias-primas levando a altos rendimentos, empregando solventes ou misturas de solventes seguros e não-tóxicos, sem degradação ou perda de compostos até hoje ainda continua a ser uma tarefa desafiadora.

Os capítulos deste trabalho fornecem uma pequena visão geral sobre as futuras perspectivas do mercado do azeite, a extensão das possibilidades de utilização dos padrões de odor de azeite, seguindo uma breve descrição das características químicas e sensoriais de azeites. Os capítulos dois e três detalham a parte experimental deste trabalho com a análise e caracterização das amostras de azeite, seguindo as tentativas de extração dos compostos voláteis responsáveis pelo seu cheiro.

Palavras-chaves: Compostos voláteis, azeite, extração com fluido supercrítico EFS, destilação, padrões de odor

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List of abbreviations

CAR	Carboxen
CLSA	Closed-loop-stripping-analysis
CE	Comissão Europeia
CO_2	Carbon dioxide
DVB	Divinylbenzene
EC	European Commission
EtOH	Ethanol
EU	European Union
EVOO	Extra virgin olive oil
Extr.	Extract
GC	Gas chromatography
GC-MS	Gas chromatography–mass spectrometry
GRAS	Generally recognized as safe
HMDS	Hexamethyldisilizane
HS-SPME	Headspace Solid Phase Microextraction
IOC	International Olive Council
MDS	Multidimensional scaling
NIST	National Institute of Standards and Technology
OAV	Odour activity value
PCA	Principal Component Analysis
PDMS	Polydimethylsiloxane
PG	Propylene glycol
PTFE	Polytetrafluoroethylene
ROO	Refined olive oil
SD	Steam distillation
SDE	Simultaneous Distillation-Extraction
SFE	Supercritical fluid extraction

SPE	Solid-phase extraction
SPME	Solid Phase Microextraction
Temp.	Temperature
TMCS	Trymethylchlorosilane
UE	União Europeia
VOC	Volatile organic compound
VOO	Virgin olive oil

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1 Introduction

Olive oil is much prized for its nutritional benefits, but the highest-quality olive oil, which is obtained from fresh and healthy fruits without solvent extraction, is also appreciated because of its unique and delicate flavour [1].

The delicate and unique flavour of olive oil is attributed to a number of volatile components. Aldehydes, alcohols, esters, hydrocarbons, ketones, furans, and other compounds have been quantitated and identified by gas chromatography-mass spectrometry (GC-MS) in good-quality olive oil, an oil with abundant flavour, the so called “virgin olive oil” [2, 3].

The presence of flavour compounds in olive oil is closely related to its sensory quality. These volatile flavour compounds are formed in the olive fruit through an enzymatic process. Olive cultivar, origin, maturity stage of fruit, storage conditions of fruit, and olive fruit processing influence the flavour components of olive oil and therefore its taste and aroma [2].

Various off-flavour compounds are formed by oxidation, which may be initiated in the olive fruit. Major compounds formed in oxidized olive oil are Pentanal, Hexanal, Octanal, and Nonanal, but 2-Pentenal and 2-Heptenal are mainly responsible for the off-flavour [2].

Sensory analysis is an essential part of evaluating olive oil quality and complements chemical analyses, which both are legal governmental requirements for determining the quality of olive oil, realised through panel tests, based on the standards of the International Olive Council (IOC) and primarily on the Regulation (EC) 640/2008 of the European Commission [4, 5, 6].

Competent, professional sensory panels are an essential tool in the production of high quality foods and beverages. The ability to describe the odour of products in objective terms and to check their conformance with pre-defined quality standards is increasingly critical to the commercial success in today’s competitive marketplace [7].

This is attributable to the fact that to the contrary as in taste, in the case of odour we have not just five qualities of perception, but instead it is possible to distinguish between a vast number of different olfactory impressions. Classifying these odour impressions in odour groups at least is difficult and needs to be trained in means to be able to build on an odour memory. Both typical positive value-adding flavours and typical off-flavours must be taken into account in product-specific odour training in purpose to build up an odour memory [8].

Through training the sense of smell and getting to know the different odour substances, it is possible to progressively achieve improvement of the odour memory so that panellists are better able to identify and describe the odours perceived in words. Defined odour standards and sensory odour descriptions building on these can support this training measure [8].

This odour memory and verbal powers of expression can be trained with the help of standardised odour references with set concentrations [8].

Often the difficulty of this type of training resides in the impossibility of obtaining reference standards that can be used by different panels, allowing to compare their performance [1].

1.1 Objectives and working plan

The objective of this work is to minimize the existing lack of olive oil odour standards for the training and monitoring of sensory analysis panels, by contributing to the preparation of these odour standards corresponding to the defects and characteristic scents, by preparation through typical and atypical olive oil samples.

For this purpose, in a first step 34 different olive oil samples with and without sensory defects, from different regions in Portugal were analysed by GC-MS. Volatile compounds of sensory relevance present in the samples were identified based on literature descriptions. In a second step, adequate extraction methods for the extraction of the volatile compounds, by applying Supercritical fluid extraction (SFE) and distillation were tested (see Figure 1).

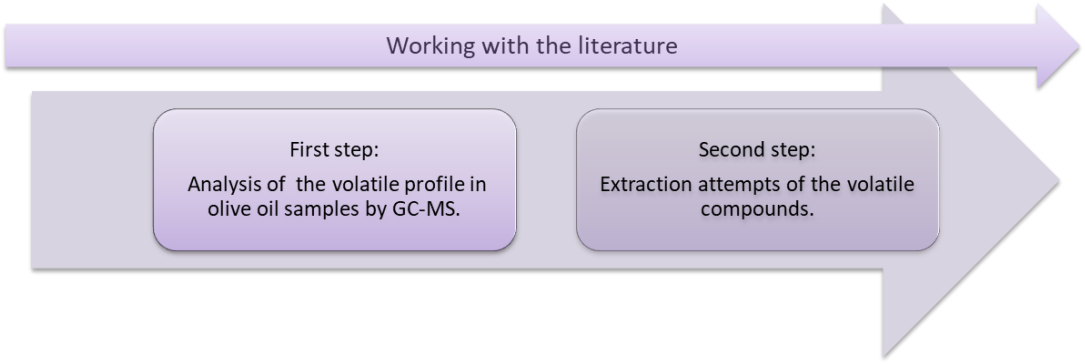


Figure 1: Overview of the process steps for the analysis and extraction of the volatile compounds for the typical and atypical odour standards.

The subsequent chapters of this work give a small overview over the market prospects of olive oil and the extent of the utilization possibilities for olive oil odour standards, following a brief description of the chemical and sensory characteristics of olive oils. Chapter two and three detail the experimental part of this work with the analysis of the olive oil samples and the extraction of the volatile compounds.

1.2 Market prospects and utilization possibilities

1.2.1 Market prospects of olive oil

Olive oil is placed as an integral component of the Mediterranean diet, and it is probably the most globally traded and consumed product that is connected to the traditional Mediterranean diet. There has been an overall increase in production of olive oil in Mediterranean countries since the 1960s [9].

By 2030, EU production of olive oil is expected to rise by 1.3% per year, driven mainly by new plantations and improvement in agronomic practices. At Member State level, these drivers include for example investment in irrigation in Spain and Portugal, improvement of the harvesting operations in Italy, along with the modernization of the milling industry in Portugal. Production is expected to increase sharply in the Iberian Peninsula (around 2 % per year, compared to the average in 2015-2017). This additional production will serve both growing world demand and increasing EU consumption, consolidating the EU's position as the biggest world producer and exporter of olive oil. Whilst in the main producing countries, i.e. Spain, Italy, Greece and Portugal it is expected that the consumption by 2030 will decrease further by 5% (compared to the average consumption of 2015-2017), due to changes in lifestyle and price increase in recent years with lower harvest. Increasing consumption outside Spain, Italy, Greece and Portugal should offset the consumption loss in these countries over the outlook period [10].

The economic importance of the Portuguese national olive oil sector has good prospects for future, although at a slower growth pace than in the last decade.

Some reasons for this are the increasing recognition of the quality of national olive oil, as well as the commercial dynamism of the main national groups of olive oil producers and distributors, who will contribute to a better appreciation of exports and, consequently, to an increasing economic weight in the national olive oil sector [11].

In a contest of increasing competition, the strategy aimed at the improvement of product’s intrinsic and perceived quality, assumes a great importance in order to obtain a competitive advantage in the markets internationalization.

The identification of different olive oil quality attributes should constitute a clear incentive for highly competitive, market-oriented firms to satisfy the needs of quality-conscious olive oil consumers [12].

Sensory analysis is an essential part of evaluating olive oil quality and complements chemical analyses, which both are legal governmental requirements for determining the quality of olive oil, realised through panel tests, based on the standards of the International Olive Council (IOC) and primarily on the Regulation (EC) 640/2008 of the European Commission [4, 5]. All olive oil production companies therefore need to have in place some form of in-house sensory testing with trained panellist.

1.2.2 Utilization possibilities

The organoleptic assessment is both a qualitative and quantitative method, since its application results in the classification of samples as extra virgin olive oil (EVOO), virgin olive oil (VOO) or lampante olive oil in accordance with Regulation (EC) 640/2008 of the European Commission, based on the median of the predominant defect and the presence or not of a fruity attribute. Consequently, tasters must be supervised for correct classification of samples and for correct recognition of the intensities of perceived attributes [6, 13].

For the olive oil industry odour reference standards could be used to:

- screen and select new tasters;
- measure and enhance the performance of individual panellists and trainees;
- measure and enhance the performance of sensory panels;
- measure and enhance the effectiveness of training programs;
- help to understand and define the sensorial quality of the products;
- or help to identify the different sensorial descriptors of the products [5, 14].

Furthermore they could be used to select and harmonize the training of potential panellists in order to guarantee sensorial acuity and the precise recognition and quantification of the characteristic attributes of the products. Otherwise, the tasters will only be able to issue general opinions that are inaccurate and of little use to anyone who wants to know specific particularities or details in order to maintain quality or improve their product according to the products typical specified characteristics [15].

The ultimate goal is to be able to establish a more effective approach to delivering consistent quality products and to better identify and promote their products which have specific characteristics [5, 14].

Given the fact that current reference materials are “natural” virgin olive oils selected for being representative of a single sensory defect, they can be slightly different year by year in sensory properties and intensity of the defect. On the contrary, a perfect reproducibility of each defect and characteristical odour would be extremely useful to align all the panels. The availability of certified reference materials, having intensity ranges for specific attributes that cover different classes of virgin olive oil, can assure correct training of sensory assessors, and is useful to determine the trueness of the evaluation carried out by the assessors (closeness to the accepted reference value as a measure of accuracy). It is particularly important to improve the sensory skills of the panel through the adoption to reference materials, built with a specific mixture of sensory relevant volatile molecules and appropriately combined in defined concentrations, also considering their odour thresholds [13].

Given the existing importance of the sensory analysis of the products in the quality control or even out of legal requirements and the existing lack in odour reference standards concerning typical and atypical aromas of the products, the formulation of these innovative odour standards to be used in the training and monitoring of sensory analysis panels will be of a great

practical convenience and benefit, in face of the vast utilization possibilities and the importance of the organoleptic assessment of olive oils.

1.3 Chemical and sensory characteristics of olive oil

It is well known, that olive oils peculiar delicious taste and aroma are dependent on its volatile compounds profile. As stated by Morales et al., the profile of these compounds is in agreement with the sensory attributes recognized and evaluated by assessors [16].

Both positive attributes and sensory defects in olive oil can be associated with volatile compounds [17].

Volatile compounds are responsible for its aroma, while phenolic compounds are related to its taste. Odour and taste joined to somatosensory information gather the complex perception of flavour [18].

The total content of volatiles in VOO is variable depending on the olive oil designation and quality. There are not one but several olive oil designations. Olive oil is marketed in compliance with the designations of the International Olive Council (IOC) trade standards and regulation of the Commission of the European Communities [6, 18, 19].

In general olive oil is defined on the basis of its sensory characteristics. European Union (EU) regulations establish the organoleptic quality of virgin olive oil by means of a panel test, evaluating positive and negative descriptors [6, 20].

Extra - virgin olive oil (EVOO) and VOO are different edible grades of VOO. Lampante VOO is not proper for consumption and is intended for refining or for technical purposes. Refined olive oil (ROO) is the oil refined by methods that include neutralization, decolourization with bleaching earth, and deodorization [18, 19].

VOO has a higher amount of total volatiles, which are produced from the olive fruit, and they are direct metabolites produced in plant organs by intracellular biogenic pathways and oxidative processes, all of them being responsible for sensory attributes appreciated by consumers [18].

The volatile content of VOO, from a qualitative and quantitative point of view, depends on various factors such as genetic characteristics, geographic origin, pedoclimatic conditions, olive ripening, processing conditions, and olive oil storage [18].

However, not all volatile compounds contribute in the same way to the sensory quality, since the contribution of these compounds depends on their concentration and on their odour threshold to stimulate and be perceived by olfactory sense and taste [16].

Differences in individual sensitivity of human subjects affect the evaluation of the odour intensity, so that the contribution of each volatiles to the oil aroma is better estimated by the odour activity value (OAV), the OAV is the ratio between the concentration and the corre-

sponding odour threshold [21]. Volatiles with OAV < 1.0 do not contribute to VOO aroma, while volatiles with OAV > 1.0 do [18].

For taste and smell sense organs depend more on chemical factors and the stereochemical structure of the molecules than on their concentrations [22].

Chemical factors such as volatility and the hydrophobic character, size, shape, conformational structure, type, and position of functional groups seem to be more associated to the odour intensity of a volatile compound than its concentration. The different nuances of green odour and the grade of pleasantness seem also to be affected by cis/trans isomerism and by the position of the double bond in volatile compounds [22, 23].

In spite of the fact that the volatile fractions of different quality virgin olive oils belong to different chemical classes, they share the characteristics described by Angerosa in Table 1 [22].

Table 1: Characteristics shared by volatile compounds responsible for virgin olive oil aroma.

- Low molecular weight (<300 Da)
- High volatility so that a suitable number of molecules can reach the olfactory epithelium as molecular dispersion, transported by the air streams due to inhalation and expiration
- Sufficient hydrosolubility to diffuse into the muxus that covers the sensitive olfactory cells
- Fair liposolubility to dissolve in membrane lipids contiguous to proteins of receptors
- Chemical features to bond specific proteins

Aldehydes, alcohols, ketones, and esters are the major compounds, but there are also furans, hydrocarbons, acids, and aromatic compounds, though in lower amounts [18].

According to Flath, Forrey and Guadagni the contribution of the non-polar fraction to the olive oil aroma is considered minimal [24].

The fragrant and unique aroma of virgin olive oils of good quality is usually described by perceptions attributable to:

1) the fruity sensation, the sensation reminiscent of healthy fresh fruit collected at the optimum of the harvesting time;

2) the sensations reminding of leaves, freshly cut grass, green fruits such as apple, banana or vegetables such as artichoke or tomato etc., accompanied by more or less intense taste notes of bitterness and pungency.

The impressions indicated under point 2) are known as “green” odour notes, and characterise the flavour of oils extracted from not completely ripe olives. They are viewed as freshness and liveliness characteristics of good quality virgin olive oils by consumers [22].

Oils from unripe fruits are characterised by quite intense green perceptions and by very high strengths of bitter and pungent attributes. On the contrary oils obtained from ripe fruits are lightly aromatic because of a low accumulation of volatile compounds that provide a typically fresh and herbal flavour, due to a reduced activity of enzymes involved in the lipoxygenase pathway. These are as well characterised by weak intensities of bitter and pungent perceptions because of a decreasing amount of phenolic compounds during the ripening of fruits. The whole of both fruity attribute and the green sensations describe the different nuances of the aroma of virgin olive oils [22].

Mainly responsible for the green positive and pleasant perceptions of the fragrant and particular aroma of virgin olive oils are C6 and C5 aromatic volatile compounds, whilst bitterness and pungency have to be mainly attributed to secoiridoid compounds [22].

On the other hand, other volatile compounds have been reported by Bendini et al. to be responsible for the unpleasant aroma and odours resulting from olive oil; these compounds can be derived from different mechanisms such as: sugar fermentation (winey), amino acid (leucine, isoleucine, and valine) conversion (fusty), enzymatic activities of moulds (musty) or anaerobic microorganisms (muddy), and other auto-oxidative processes (rancid) [23, 25].

The International Olive Oil Council has developed a specific vocabulary for virgin olive oil sensory descriptors. In these, fruity, bitter and pungent are considered as positive attributes whereas the common defect attributes are fusty/muddy-sediment, musty-humid-earthy, winey-vinegary, acid-sour, rancid and frostbitten [26].

It is important to mention that the relationships of the sensory sensations do not always result from the interactions between a single odour note and a single volatile compound, but from the connections between a single attribute and the totality of the volatile compounds or from the percieved fusion and blending of taste and odour sensations which could give rise to new qualities [22].

The sensory evaluation detects oxidative deterioration before changes are observed in parameters like the free fatty acid level or peroxide value, this emphasizes the importance of volatile compounds in detecting early stages of olive oil deterioration [27].

Concerning quality indices, it must be said that in the production of EVOO it is easy to fulfil the commercial category specifications for chemical quality characteristics like the free fatty acid or peroxide value, but it is more difficult to reach the required absence of the median of a defect in the organoleptic evaluation [28].

2 Analysis of olive oil samples by GC-MS

For the analysis of the volatile compounds present in the 34 olive oil samples Head space solid-phase microextraction (HS-SPME), coupled to GC-MS was used.

HS-SPME is a rapid, solventless sampling procedure which, combined with GC-MS analysis is a useful method for the analysis of volatile compounds. In this efficient, cheap, and simple technique, a polymeric film coated on a fiber is exposed to the gas phase that lies immediately over the solid or liquid sample, as illustrated in Figure 2 [1, 29].

The polymer coating acts like a sponge, concentrating the volatile analytes by absorption/adsorption processes. After the sampling, the fiber is retracted into the metal needle and the next step is the transfer of the analytes by desorption through high temperature exposure into the chromatograph, as the injector port is at a high temperature [18].

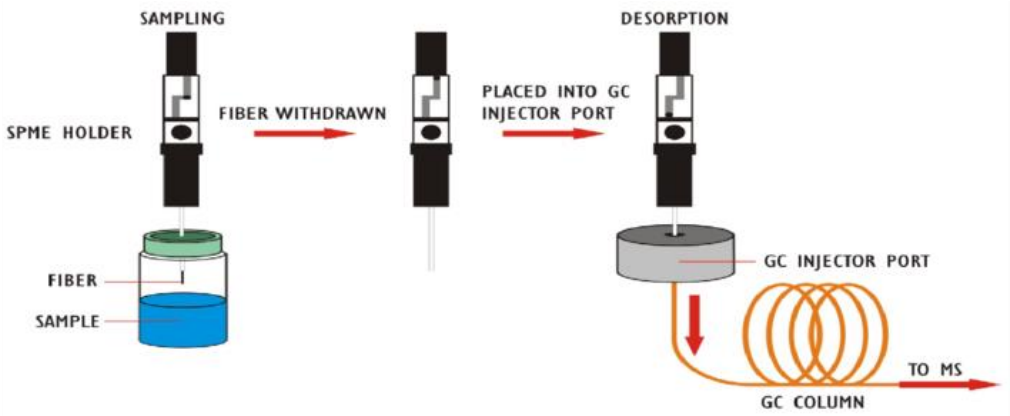


Figure 2: Diagram of analysis with solid-phase microextaction gas chromatography-mass spectrometry (HS-SPME-GC-MS).

This operation strategy has the advantage of being a non-destructive technique and allows the evaluation of the samples at different experimental conditions [1, 29].

SPME has been profusely applied in the literature for the analysis of VOO volatile compounds [1, 29]. Different analysis conditions were tested with the objective to optimize the analysis conditions for the characterization of the volatile compound profiles of the 34 different olive oil samples.

2.1 Sample analysis: equipment and materials

The following equipment and materials were used for the olive oil analysis:

Table 2: GC-MS Sample analysis: equipment and materials.

Analytical Balance	Santorius Lab Instruments GmbH & Co. Kg. ENTRIS224 - 1S
GC-MS	GC-MS used with Wax column: QP 2010, Shimadzu with AOC-5000 Autosampler GC-MS used with ZB-5 column: QP 2010 Plus, Shimadzu with AOC-5000 Autosampler
SPME fiber	(DVB/Car/PDMS) fiber 50/30µm, 2cm length (SUPELCO Analytical, Bellefonte, PA, USA)
Analytical columns	Wax column: Sapiens – Wax MS (Teknokroma), 60 m, 0.25 mm (IS), 0.25 µm (film thickness) ZB-5 column: ZB-5MSi (Zebron), 30 m, 0.25 mm (d.i.), 0.25 µm (film thickness)
Carrier gas	Helium Purity ≥ 99,999%
Headspace glass vial	20 ml volume with aluminum cap and PTFE / silicone septum
Olive oil samples	34 portuguese olive oil samples without (21) and with (13) sensory defects

2.2 Sample preparation and analysis conditions

Sample preparation is an essential step in the analysis of aroma compounds in VOO, greatly influencing the precision and accuracy of the results and the time and cost of the analysis [34]. HS-SPME has been extensively used by various authors for the analysis of olive oils. Headspace to olive oil ratio used by authors in literature vary a lot [31-36].

Kalua et al. developed a HS-SPME method for monitoring volatile compounds in extended time–course experiments and determined a Headspace/olive oil ratio of 1g oil in 10ml vial to give the best results [37].

Romero et al. used for the validation of a SPME–GCMS method for the analysis of virgin olive oil volatiles responsible for sensory defects a ratio of 2g sample for a 20ml glass vial [35].

In this experiment 6,5g of olive oil sample were weighted in a 20ml vial, based on previously empirically established experience by GC-MS operator. Vials were closed with screwcap and septum and placed on GC-MS autosampler.

The SPME technique was performed automatically using a DVB / CAR / PDMS fiber exposed to the headspace of the vial containing the sample according to the conditions defined in Table 3.

Table 3: Analysis conditions by SPME.

SPME	Extraction temperature	40 ºC
	Stirring Speed	250 rpm
	Extraction time	40 min
	GC desorption time	6 min

GC-MS operating conditions are summarized in Tables 4 and 5.

Table 4: GC-MS analysis conditions.

GC-MS	Carrier gas/flow rate	Helium / 2 mL/min
	Injector Temperature	250 ºC
	Injection Mode	Splitless
	Split ratio	N/A
	Ion source temperature	250 ºC
	Detector Temperature	250 ºC
	Analysis Time	37 min

Table 5: GC-MS chromatographic program.

Speed (ºC / min)	Final temperature (ºC)	Hold (min)
	40.0	5.00
5.00	170.0	0.00
30.00	230.0	4.00

Evaluations done with diferent types of fibers reported DVB-CAR-PDMS fiber to be the most suitable for analysis of volatile compounds in virgin olive oil [1, 36-39].

As this type of fiber covers a wide spectrum of volatile compounds present in olive oil. The major volatile compounds in fresh virgin olive oil are reported to be C5 and C6 compounds (such as (E)-2-Hexenal), whereas oxidised oil shows increasing amounts of C7–C12 compounds (e.g. Nonanal). To detect such a wide spectrum of compounds, a divinylbenzene-carboxen-polydimethylsiloxane (DVB-CAR-PDMS) fiber coating was used. Mixed coatings with DVB are suitable for volatile compounds analysis as DVB reduces molecular weight discrimination. The CAR coating has a high sensitivity for small volatile molecules while the PDMS has very high sensitivity to non-polar compounds. The choice of DVB-CAR-PDMS fiber offers both broad linear concentration range and low detection limits [37].

As the DVB-CAR--PDMS fibers show to have the best efficiency in sampling the volatiles present in VOOs, no other fibers in this work were tested [1, 36].

Analysis conditions by SPME in Table 3 were chosen on basis of the alterations and optimizations of the method done in Chapter 2.3 and 2.4.

Volatile compounds detected on chromatograms were identified using the NIST 21, 27, 107, 147 and Wiley 229 mass spectra libraries. The identification of the compounds with sensory relevance was done through research in the existing literature. Kovats retention indices were confirmed for the compounds of sensory relevance by comparing with those reported in literature. All of the 34 olive oil samples were analysed in triplicate.

2.3 Alterations and optimization of the analysis conditions

To optimize the analysis conditions for the characterization of the volatile compound profiles of the 34 different olive oil samples, in a first step a commercially available olive oil was analysed in duplicate, altering SPME standard conditions shown in Table 6 simultaneously in a ZB-5 semi-standard non-polar column and in a standard polar DB-Wax column.

Table 6: Normal / Standard SPME conditions.

Fiber conditioning	3min, 250°C
Fiber extraction and adsorption program	40°C, 40min
Fiber desorption time to column	3min

After analysing in standard conditions, analysis conditions were changed once on both columns:

1. Changing only the fiber extraction and adsorption time from 40 to 30 minutes.

2. Changing only the fiber desorption time to column from 3 to 6 minutes.

3. Changing only the adsorption temperature from 40° to 45°C.

The best analysis conditions were chosen as by total number of compounds identified, number of compounds with sensory relevance identified and chromatograms with highest peak areas.

2.3.1 Results and conclusion

Table 7 shows the results of the comparison between the different analysis conditions applied on the ZB-5 and on the Wax column. It is clearly visible that by the total number of compounds identified and by the number of compounds identified with sensory relevance; the more polar Wax column with 25 compounds identified with sensory relevance is the choice to use for the volatile compound characterisation of the 34 olive oil samples. This result stands in line with the findings of Flath, Forrey and Guadagni as mentioned in Chapter 1.2 about the chemical and sensory characteristics of olive oils, who considered the contribution of the non-polar fraction of the olive oil to its aroma as minimal and thus didn't examine these more extensively, as in this experiment way more compounds were able to be detected and identified with the more polar Wax column [24].

Table 7: Results comparison between different analysis conditions (ZB-5 and Wax column).

Column	Conditions	No. Total compounds	No. compounds with sensory relevance
ZB-5	Normal	51	7
	Extraction time 40min → 30min	24	7
	Desorption time 3min → 6min	43	9
	Adsorption temperature 40°C → 45°C	44	7
Wax	Normal	169	**25**

The identification of the compounds with sensory relevance was done through an extensive research in the existing literature. The preferable choice for the Wax column over the ZB-5 column becomes more evidential by looking at chromatograph comparison shown in Figure 3. For both columns were used the same analysis conditions and program as mentioned in Chapter 2.2, altering the desorption time for both to six minutes.

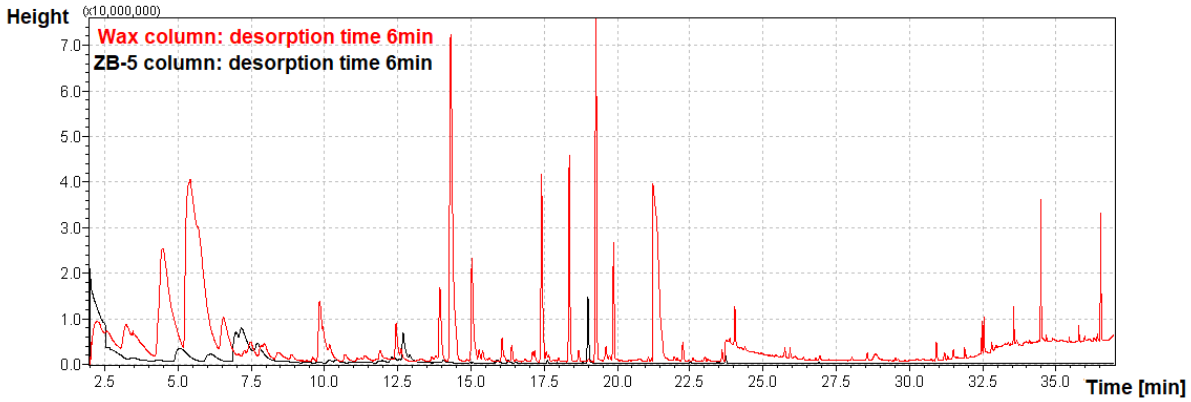


Figure 3: Comparison of chromatographic results red coloured chromatogram: Wax column at desorption time of six minutes. In comparison to black coloured chromatogram: ZB-5 column at desorption time of six minutes.

As shown in Figure 4, listed below, by overlaying the different chromatograms of the Wax column, altering the analysis conditions for the Wax column did not make significant difference, in exception of a bit larger peak in (E)-2-Hexenal and higher peak areas in carboxylic acids appearing at the end of the chromatogram, using an adsorption temperature of 45°C or the normal conditions. The latter higher peaks, marked in the chromatogram are likely to stem from oxidative alteration of the sample, rather than from a higher extraction efficiency [39, 67]. Nunes et al. evaluated the effect of heating on the volatile composition of extra-virgin olive oil and reported higher peaks in carboxylic acids in olive oils heated at 150 and 200°C, what furthermore affirms this proposition [49].

As the softer extraction conditions obtained volatile compounds more representative of the virgin olive oil flavour and regarding that on the ZB-5 column it was obtained a higher amount of compounds identified with a desorption time of 6 minutes from fiber to column (see Table 7), it was decided to analyse the 34 olive oil samples parallelly on both columns using a desorption time of six minutes from the fiber to the GC-column. Nevertheless for future analysis in this work only analyses from the Wax column were taken into consideration, as they resulted by far in the best analysis results.

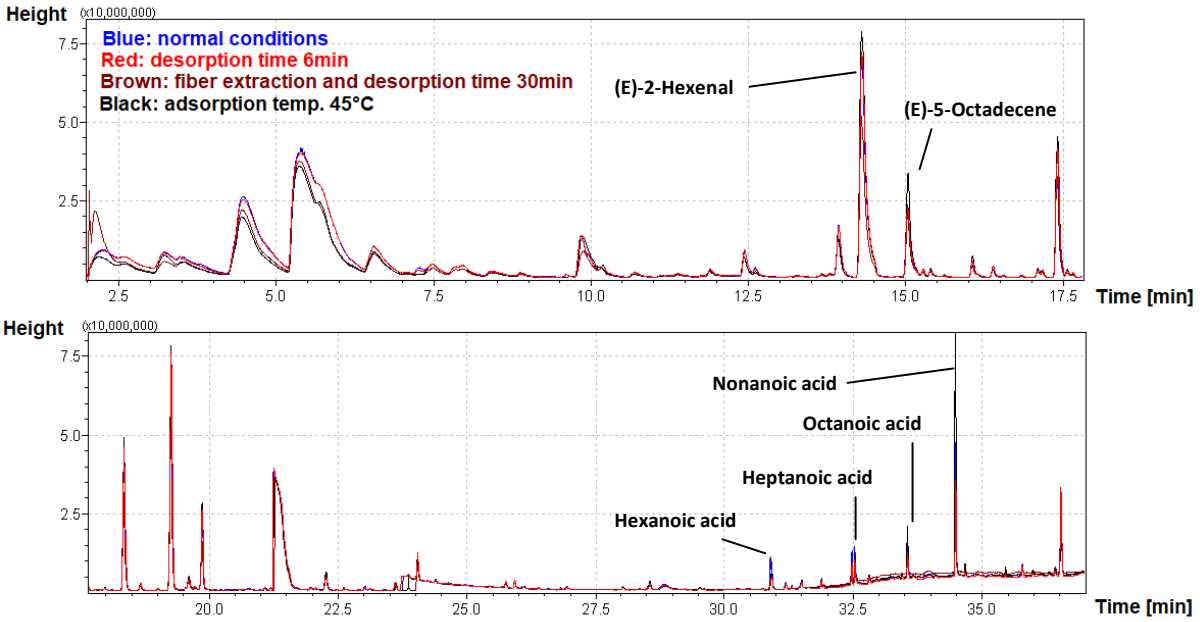


Figure 4: Comparison of chromatographic results on Wax column blue coloured chromatogram: normal conditions (see Table 6). Red: desorption time of six minutes. Brown: Fiber extraction and adsorption time 30 minutes. Black: adsorption temperature 45°C.

2.4 Results and discussion

34 olive oil samples were analysed with conditions described in Chapter 2.2.

Analysis results show that in average 172 compounds from the 34 samples have been separated by HS-SPME-GC-MS analysis from the volatile fraction, as can be taken from Table 8.

In average 51 of these, per sample, could be identified through literature research being of sensory relevance.

Reviews in literature using GC-MS, list around over 100, up to approximately 180 compounds belonging to several chemical classes (aldehydes, alcohols, esters, ketones, hydrocarbons, acids) which have been separated from the volatile fractions of EVOOs of different quality [23, 40, 41].

Table 8: Analysis results of 34 olive oil samples, 21 without and 13 with sensory defect.

Sample (red = defective)	N° Compounds	N° compounds not identified	N° compounds of sensory relevance	Notes
A	285	82	59	EVOO
B	218	40	54	EVOO – Varieties: Koroneiki, Cobrançosa
C	191	25	50	EVOO – Declared as “green fruity aroma with a mild bitter and spicy at the end.” Variety: Cordovil
D	179	17	51	EVOO – Declared as “ripe fruity aroma, leaving a sweet sensation with a mild spicy at the end.” Variety: Galega

Sample (red = defective)	N° Compounds	N° compounds not identified	N° compounds of sensory relevance	Notes
E	143	11	51	EVOO – Declared as “fruity”. Varieties: Frantoio, Cobrançosa, Arbequina
F	131	6	45	EVOO
G	189	23	55	EVOO – Varieties: Galega, Cobrançosa
H	173	24	50	EVOO – Declared as “fruity, medium ripe and mild bitter and spicy”
I	178	11	52	EVOO – Varieties: Madural, Cobrançosa, Verdeal
J	180	14	50	EVOO – Best before date: 07/2014, flask was already open upon delivery. Variety: Picual
K	161	4	55	EVOO – Best before date: 07/2015, flask was already open upon delivery. Variety: Cobrançosa
L	171	9	50	EVOO – Best before date: 07/2015, flask was already open upon delivery. Variety: Blend
M	180	14	59	EVOO – Best before date: 07/2014, flask was already open upon delivery. Variety: Maçanilha
N	165	16	51	EVOO, flask was already open upon delivery. Variety: Verdeal
O	166	10	50	EVOO – Best before date: 07/2014, flask was already open upon delivery. Variety: Cobrançosa
P	152	14	48	EVOO
Q	164	14	54	EVOO – Declared as “Flavour: mild, sweet and with nuttz notes”
R	151	16	50	EVOO – Declared as “Flavour: fresh, fruity and slightly spicy”
S	159	12	50	EVOO – Tasting Note: Fresh, fruity and creamy with aromas and flavours of tomato, apple, lemon and almond and with a slightly spicy finish. Varieties: Cobrançosa, Arbequina
T	153	17	51	–
U	172	13	50	EVOO – Best before date: 07/2014, flask was already open upon delivery
V	190	14	52	EVOO – Best before date: 07/2014, flask was already open upon delivery
X	142	16	49	–
Z	154	21	52	Variety: Sikitita
AA	146	13	48	Variety: Picual
BB	145	14	55	Variety: Cobrançosa
CC	138	16	52	Variety: Arbosana
DD	165	16	52	Variety: Galega
EE	140	19	51	Variety: Arbequina
FF	192	19	50	–
GG	189	28	51	–
HH	189	33	52	–
II	193	23	48	–
JJ	192	29	47	–
	Ø 172	Ø 19	Ø 51	

Principal component analysis (PCA)

In order to better point out and explore the relationships and differences between the volatile compounds identified and the characteristic volatile profiles of each of the 34 olive oil samples analysed, principal component analysis (PCA) was carried out.

The use of exploratory and classification statistical approaches such as principal component analysis (PCA) can identify patterns in samples and variables contributing to the clustering of samples. In order to find homogeneous groups of olive oil samples according to the studied factors [27].

In a first step for this purpose, percentual peak areas and volatile compounds with their respective retention times for each of the chromatographic triplicates of the 34 olive oil samples were determined and identified.

In a second step the average retention times and average percentual peak areas of the identified volatile compounds of the triplicates were calculated and the results for each of the 34 samples aligned together in one excel sheet, corresponding to their equal retention times.

In a third step, after an initial treatment of the variables, removing variables with non-significant percentage of peak area, it was possible to receive a complete graphical model that allowed to determine which of the volatile compounds are more determinant or stand out in means of peak area for each olive oil sample, using the statistical analysis software The Unscrambler X.

The PCA results are graphically displayed using two plots.

In the first one, the sample scores are plotted to show the relationship between the samples (Figure 5); in the second (Figure 6), the volatile compounds are plotted to aid the interpretation of principal components in terms of the volatile compounds. The two plots can be interpreted together (Figure 7). For the purpose of better exploring and pointing out differences and relationships in the sensory characteristical profile between the samples, only compounds with sensory relevance identified through research in the literature (see Table 9) were considered for PCA.

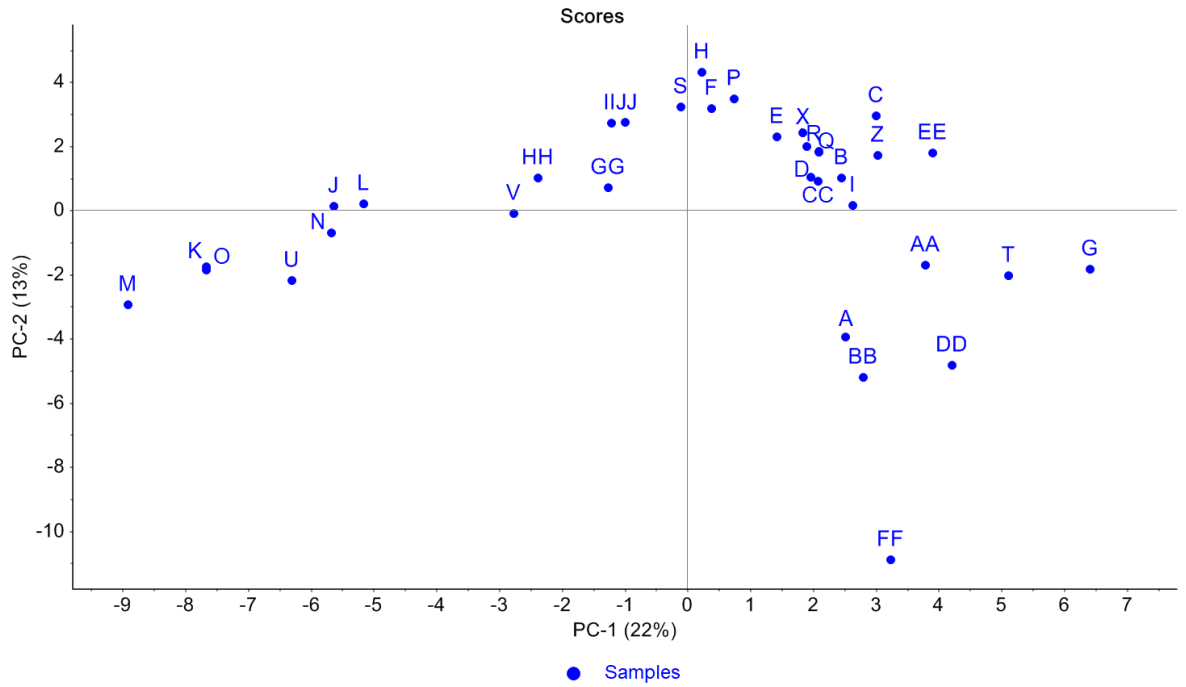


Figure 5: PCA olive oil sample scores are plotted to show the relationship in terms of VOCs (similarities and differences) between the samples.

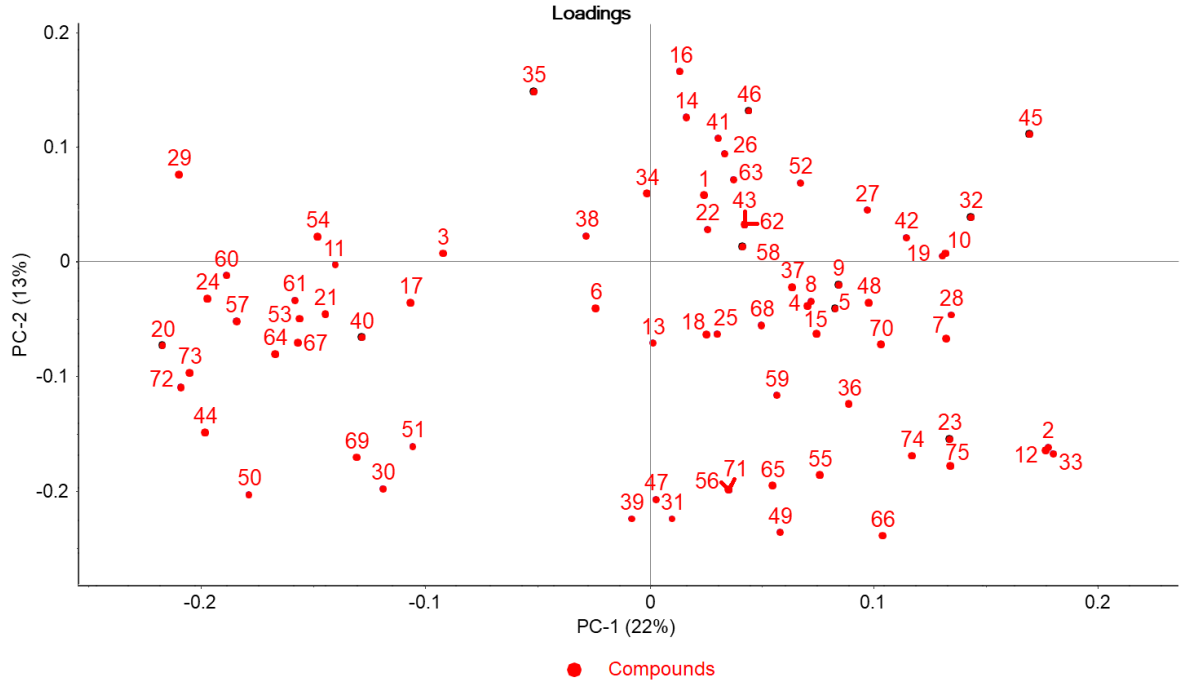


Figure 6: PCA volatile compounds are plotted to aid the interpretation of principal components in terms of the volatile compounds with sensory relevance. Corresponding compounds to the numbers are listed in Table 9.

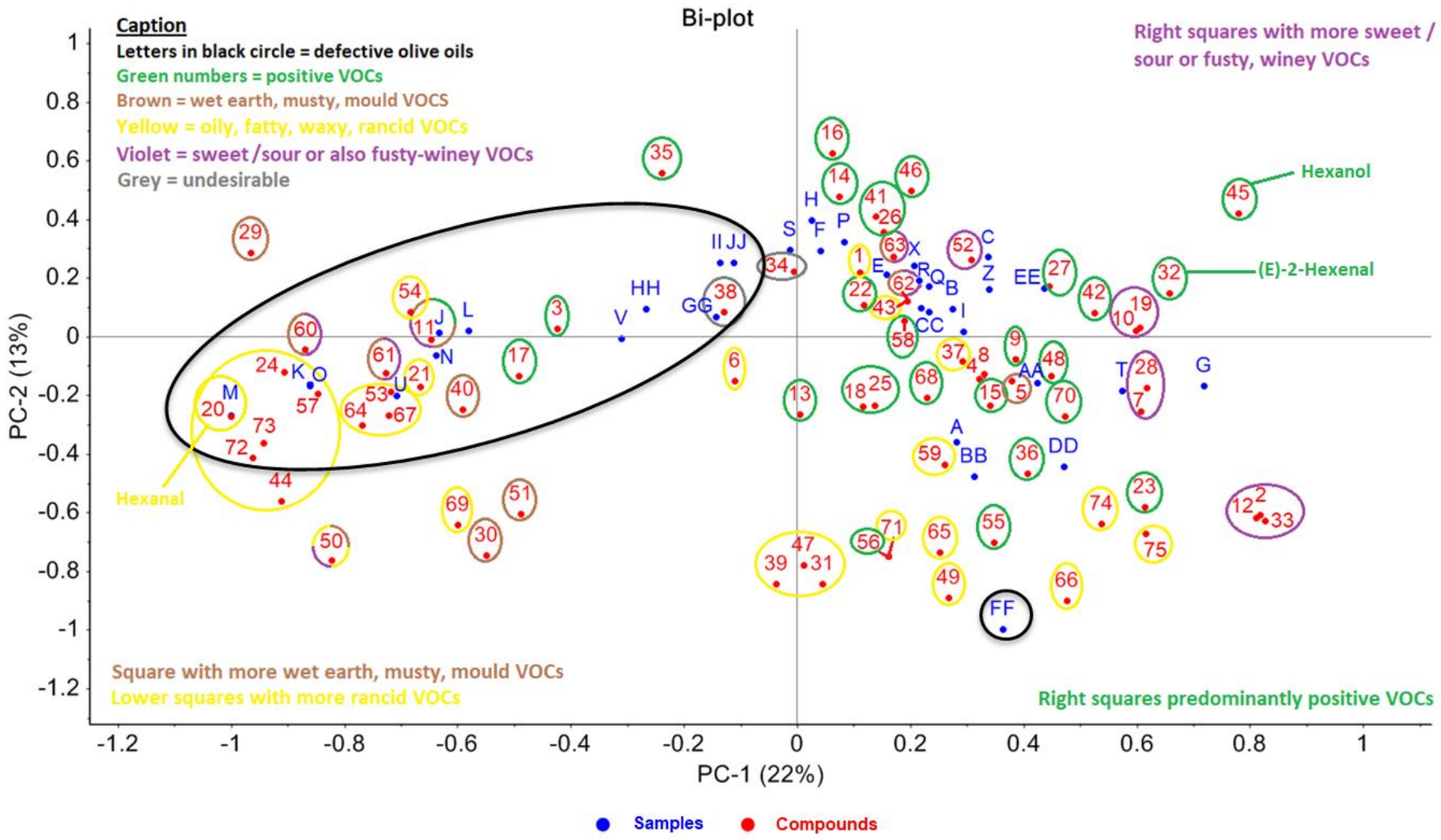


Figure 7: PCA volatile compounds (principal components) plotted together with the samples to aid the interpretation of the relationship between principal components in terms of the percentual peak areas of volatile compounds and the olive oil samples.

Principal compounds shown in Figure 7 were marked with coloured circles for better illustration and related to their respective principal sensory perception marked with the same colour, as can be taken from the caption inside Figure 7.

For reasons of simplification, as green marked compounds were considered to be volatile compounds with positive contribution to the sensory profile, while brown, yellow, violet and grey groups were considered to be VOCs contributing negatively to the sensory profile and positively to the sensory defects, based on findings in the literature. In some cases their positive or negative contribution to the sensory profile though depends not solely on their presence, but also on their concentration and odor threshold. Negative aromas such as rancid, fusty, winey-vinegary, and musty-humid earthy are sensory attributes of defective virgin olive oil recognized by the International Olive Council [19].

Table 9 shows the respective principal compounds associated to score numbers in the PCA plot.

Table 9: Respective principal compounds and their sensory perception, associated to score numbers in PCA plot.

Score plot number	Principal compound and sensory description	Colour code	References
1	Pentanal: oily, wood, bitter, almond / Almond, malt, pungent (rancid, oxidized)	Yellow	1, 35
2	2-Methylbutanal: Malty / correlated with fusty defect	Violet	1, 38
3	(Z)-2-Penten-1-ol: Banana (sniffing), green (grass) (MDS)	Green	2, 54, 46
4	Acetaldehyde: Pungent, sweet, floral	-	38, 82
5	Dimethyl sulfide: Organic, wet earth	Brown	38
6	Propanal: Sweet, pungent, floral (oxidation)	Yellow	38, 41, 97
7	Octane: alkane, sweet, solvent - correlated to fusty and winey defects	Yellow	1, 52
8	2-Methyl-propanal: Cooked, caramel	-	38
9	Methyl acetate: Green / green (nuts) (MDS) / Ester	Green	1, 2, 38, 54
10	Ethyl acetate: Sweet, aromatic (sniffing), slightly bitter/pungent (MDS) / (Fusty, winey–vinegary, undesirable)	Violet	1, 2, 50
11	2-Butanone: Fragrant, pleasant (sniffing), tomato, apple (MDS) / (Pleasant, muddy, fusty)	Mixed	2, 34, 50, 97
12	3-Methylbutanal: Sweet, fruity (sniffing), ripe fruit (sensory wheel) / (fusty)	Violet	1, 55
13	Ethanol: Alcoholic, ripe apple, floral / ethanol and ethyl acetate, greater than their sensory thresholds: winey attribute	Violet	22, 38

Score plot number	Principal compound and sensory description	Colour code	References
14	2,4-HEXADIENAL: Ripe fruit, cut grass / (E,E)-2.4-hexadienal: Fatty, solvent	Green	1, 2, 38, 55
15	2-Hexenal: Sweet, fragrant, almond, fruity, green, leafy / Freshly cut grass, banana (desirable)	Green	1, 34, 50
16	3-Pentanone: Sweet (sniffing), green (sensory wheel) / Fruity, green, sweet	Green	1, 2, 47
17	1-Penten-3-one: Sweet, strawberry (sniffing), sweet (sensory wheel) / tomato	Green	2, 54, 98, 99
18	Methylbenzene / Toluene: Glue, solvent-like (sniffing), ripe fruit (sensory wheel)	Green	1, 2, 55
19	3-methylbutanoic acids: Fusty defects	Violet	1
20	Hexanal: Oily, fatty, green, green apple, lawn	Yellow	1, 44, 53
21	Decanal: Penetrating, sweet, waxy, painty / Fatty, soapy / Rancid (undesirable)	Yellow	1, 34, 53
22	2-Methyl-2-butenal: Solvent-like (sniffing); ripe fruit (olives, dry wood) (MDS) / Apple	Green	38, 54
23	2-Methyl-1-propanol: Ethyl acetate-like (sniffing), green (sensory wheel)	Green	2, 54, 55
24	(E)-2-Pentenal: Green, apple (sniffing), ripe fruit (soft fruit) (MDS)	Green	2, 54
25	Ethylbenzene: Strong (sniffing), bitter taste (dried green herbs) (MDS) / Fruity (sensory wheel)	Green	2, 54, 55
26	(Z)-3-Hexenal: Green leaves, grassy, green, apple-like, leaf-like, cut grass	Green	1, 2, 38
27	2-Methyl-4-pentenal: Dried leaves (sniffing), bitter-pungent (sensory wheel)	Green	1, 55
28	1-Butanol: Winey	Violet	35, 51
29	1-Penten-3-ol: Wet earth (sniffing), undesirable (sensory wheel) / Lawn, olive, leaf, pungent	Brown	1, 2, 47, 54, 55
30	2-Heptanone: Sweet, fruity, cinnamon (mustiness-humidity)	Brown	1, 35, 82
31	Heptanal: Oily, fatty, heavy, woody, penetrating, nutty / Greasy rancid / Fatty, citrus, rancid	Yellow	1, 34, 35, 51, 52
32	(E)-2-Hexenal: Bitter, almonds, green-fruity (sniffing), bitter (sensory wheel)	Green	35, 54, 55
33	3-Methyl-1-butanol: Winey–vinegary, fusty (undesirable)	Violet	2, 50, 54
34	Dodecene: Undesirable (sensory wheel) / Slightly bitter-taste (MDS)	Grey	2, 54, 55
35	1-Pentanol: Fruity, strong, sticky, balsamic	Green	47, 100

Score plot number	Principal compound and sensory description	Colour code	References
36	3-Octanone: Nut	Green	35, 51
37	1,2,4-Trimethylbenzene: Fish oil, unpleasant (sniffing), undesirable (sensory wheel)	Yellow	55, 84
38	2-Octanone: Undesirable / Mould, green	Grey	46, 47
39	Octanal: Fatty, sharp, citrus-like, soapy	Yellow	2, 47, 101
40	1-Octen-3-one: Mushroom, mould, pungent; Mushroom-like (rancid)	Yellow	2, 82, 101
41	(Z)-2-Hexen-1-ol: Green fruit (sniffing), green (sensory wheel)	Green	1, 3, 55
42	(E)-2-Hexen-1-ol: Green, grassy, sweet, leaves	Green	47, 102
43	(E)-2-Heptenal: Oxidised, tallowy, pungent / Soap, greasy, almond, pungent / Chemical, fatty	Yellow	52, 79, 82
44	6-Methyl-5-hepten-2-one: Fruity (sniffing), bitter taste (dried green herbs) (MDS) / (mustiness-humidity, fusty, muddy)	Mixed	1, 2, 53, 103
45	1-Hexanol: Fruit, banana, soft, grass	Green	38, 47
46	(E)-3-Hexen-1-ol: Fruity, fatty, pungent, cut grass / Green leaf, nuts	Green	2, 38, 54
47	(E,E)-2.4-Hexadienal: Fatty, solvent	Yellow	79
48	(Z)-3-Hexen-1-ol: Banana (sniffing); Green banana (MDS)	Green	2, 55
49	Nonanal: Rancid, fatty, waxes, pungent, citrus	Yellow	1, 52
50	(E)-2-Octenal: Herbaceous, spicy / Fatty	Yellow	47, 101
51	1-Octen-3-ol: Mushroom, mouldy / Musty–humid undesirable	Brown	35, 50, 52
52	Acetic acid: sour, vinegary / Winey / (Undesirable)	Violet	1, 52, 55
53	2,4-Heptadienal: Fatty, rancid, cinnamon	Yellow	34, 82, 43
54	(E,E)-2,4-Heptadienal: Fatty, rancid	Yellow	47
55	alpha-Copaene: Sweet, fruity	Green	79, 78
56	(E)-2-Hepten-1-ol: Floral	Green	79
57	3,5-Octadien-2-one: Fatty, fruity	Yellow	1, 3
58	Benzaldehyde: Almond	Green	38
59	(E)-2-Nonenal: Paperlike, fatty, sharp, cut grass	Yellow	38, 104
60	Propanoic acid derivate 1: Pungent, sour, mould	Violet/ Brown	1, 18, 52, 53, 82
61	Propanoic acid derivate 2: Pungent, sour, mould	Violet/ Brown	1, 18, 52, 53, 82
62	Propanoic acid derivate 3: Pungent, sour, mould	Violet/ Brown	1, 18, 52, 53, 82
63	Propanoic acid derivate 4: Pungent, sour, mould	Violet/ Brown	1, 18, 52, 53, 82

Score plot number	Principal compound and sensory description	Colour code	References
64	Butanoic acid: Rancid, cheese, sweat	Yellow	47, 53, 43
65	(E)-2-Decenal: Painty, fishy, fatty	Yellow	53, 82
66	1-Nonanol: Fatty, rancid	Yellow	47, 82, 43
67	(E,E)-2,4-Nonadienal: Soapy, penetrating; Deep-fried / Fatty	Yellow	53, 101, 82, 79
68	Valencene: Mint, orange blossom / Green, oil	Green	79, 105
69	Hexanoic acid: Pungent, rancid, sweaty	Yellow	47, 53
70	alpha-Farnesene: Floral, green plant	Green	79
71	2-Undecenal: Fresh, fruity, orange peel	Yellow	1, 41, 79
72	(E,Z)-2,4-Decadienal: Deep-fried	Yellow	2, 47, 82
73	2,4-Decadienal: Powerful, fatty, citrus	Yellow	1, 34
74	Heptanoic acid: Rancid, fatty	Yellow	53, 82
75	Octanoic acid: Rancid, fatty / Oily, fatty	Yellow	47, 53

Yellow marked compounds: rancidity and oxidation

Figure 7 shows that the PCA plot allowed the separation of samples with sensory defect of the ones presenting no sensory defect. As the defective marked samples were strongly characterized by higher values of compounds related to rancid olive oil and markers related to off-flavours of virgin olive oil oxidation. These compounds and samples are found more concentrated in the extreme left part of the PCA plot, correlated to compounds like Hexanal (**20**), (E,Z)-2,4-decadienal (**72**), 2,4-Decadienal (**73**) and (E)-2-Pentenal (**24**), amongst others.

Sample M strongly correlated to Hexanal (**20**). This sample together with sample U of all 34 samples presented the highest peak area in this compound. Hexanal (**20**) contributes to the perception of a sweet-green sensory note in EVOO when its concentration is higher than its odour threshold, but it contributes to the rancid perception when it is present at high concentrations [1]. Characteristically it was possible to distinguish all of the 13 sensory defective samples by having higher peak areas in this compound, than the regular samples that presented no sensory defect.

Aliphatic ketones like 6-Methyl-5-hepten-2-one (**44**) and 3,5-Octadien-2-one (**57**) are formed by autoxidation of unsaturated fatty acids and also contribute to the undesirable flavours of the oxidized VOO samples, because they have low threshold values. These ketones are characterized as having fatty, fruity odour notes [1]. The latter one, together with (E)-2-Pentenal (**24**) highly correlated with samples K and O. (E)-2-Pentenal is another common marker to find in literature for olive oil oxidation [42].

Eye-catching is the sample FF which appears alone as defective olive oil in the lower right square of the PCA plot. This sample correlates with Nonanal (**49**), Nonanol (**66**) and (E)-2-Decenal (**65**), but also with the compound alpha-Copaene (**55**), characterized positively as sweet, fruity. The first three ones although are strongly correlated to a rancid defect [43].

Compound Nonanal (**49**) appears in a much lower amount in the defective samples, concentrated at the left squares of the PCA plot, in comparison to the sample FF in the lower right square. Whilst compound Nonanol (**66**) does not appear at all in the samples concentrated in the left squares, with exception of GG in a low amount. Several papers suggest the detection of Nonanal as an appropriate method to detect initial oxidation [41, 42, 44, 45].

Samples BB, DD and A with no sensory defect presented higher peak areas in Nonanal (**50**), what according to this plot and the authors findings could suggest an progressive initiation of these samples towards oxidation.

The defective samples GG and HH were the only samples with peak areas with an insignificant amount of 2-Octanone (**38**), which is described in literature as undesirable, mould and green [46, 47].

During the sample preparation of the vials for GC-MS analysis it was remarkably noticeable that the defective samples FF, GG, HH, II and JJ were less characterized by an off-flavour odour note, in comparison to the rest of the defective olive oil samples, which affirmatively to this fact did concentrate more onto the extreme left edge of the PCA plot, confirming a apparently higher oxidation degree already noticed by smelling on the samples, based on the volatile compounds peak areas.

Green marked compounds: positive attributes

While the defective samples concentrated more on the left extreme edge of the PCA plot, the samples without sensory defect were concentrated more in the upper right square of the PCA plot, close to the center of the PCA plot.

C6 aldehydes like Hexanal (**20**), (E)-2-Hexenal (**32**), as well as Hexanol (**45**), contribute to the typical green sensory attributes which characterize EVOOs [35, 48]. According to Nunes et al. (E)-2-Hexenal is the most abundant volatile compound in European, Tunisian and Moroccan olive oils [49]. In fact, (E)-2-Hexenal (**32**) showed to be the most abundant compound by means of peak area in the samples without sensory defect.

Looking at the PCA plot it is recognizable that most of these defect free olive oil samples did concentrate between the three in beforehand mentioned positive correlated volatile compounds Hexanal (**20**), (E)-2-Hexenal (**32**) and Hexanol (**45**). It could be deduced that conforming the oxidation degree increases, the olive oil samples move from compounds **45** and **32** in the upper right part of the PCA plot, gradually more towards in direction to compound **20** in the outer edge of the left skirt of the PCA plot. Implying a direct relationship between the concentrations of these compounds.

In fact according to the literature the Hexanal/(E)-2-Hexenal ratio is a very important indicator of the freshness of the oils and can be used to estimate their oxidation degree. High quality oils show higher (E)-2-Hexenal levels than Hexanal. When oil oxidation is induced, a fast increase of Hexanal and a decrease of (E)-2-Hexenal levels takes place, and then a “rancid” off-flavour appears [48].

This fact is well demonstrated by the PCA plot. Whilst Hexanal (**20**) shows to be the main qualifier and driving force for the oxidized olive oil samples, showing higher peak areas for this compound in oxidized samples and lower peak areas in (E)-2-Hexenal (**32**) and Hexanol (**45**), the opposite seems to appear for the olive oil samples without defect. Leading to distinguish the samples with and without sensory defect in clear different groups.

Violet marked compounds: fusty-winey

Another distinguishable group are VOCs related to fusty or winey off-flavours. This group mainly concentrates on the skirts of the right squares on the PCA plot.

Especially sample G presents higher values in Octane (**7**) and Butanol (**28**), but also in compound Ethyl acetate (**10**), the last one together with sample T. These compounds amongst others are related to as fusty, winey-vinegary described off-flavours [1, 50, 52]

Whilst most of the as with sensory defect marked samples which concentrated more on the left squares of the PCA plot, presented very low or none at all values in most compounds related to a fusty winey defect, the defective sample FF presented higher values in these. What could explain its deviation from the other defective samples.

An exception in this case are the four derivates of Propanoic acid in the plot (**60, 61, 62, 63**).

Derivates **61**, **62** and **63** present negligible concentration of Propanoic acid. Only derivate **60** presents higher concentrations for the samples J, L, N and HH. It has to be mentioned that this compound can be related to an as fusty, but also as fusty-muddy or sour-mould defect [1, 18, 52, 53].

Brown marked compounds: wet earth, musty, mould

As can be taken from the PCA plot in Figure 7, not many compounds related to a musty, humid off-flavour appear in the 34 samples. Their presence in the samples is only characterized by trace values in the compounds 2-Heptanone (**30**), 1-Octen-3-one (**40**) and 1-Octen-3-ol (**51**). And therefore should not contribute much to the overall sensory perception of the samples.

1-Penten-3-ol (**29**) shows significant higher peak areas in the sensory defective samples, in comparison to samples without defect. This compound is described as undesirable and having a wet earth smell [46, 54, 55]. According to Morales and Przybylski aliphatic alcohols like 1-Penten-3-ol make a small contribution to off-flavours because their flavour thresholds are significantly higher than those of their aldehyde counterparts [1].

These compounds are more concentrated in the left squares of the PCA plot, close to the sensory defective samples. Exceptionally Dimethyl sulfide (**5**) appears in the right square of the PCA plot, this is attributable to sample G, which presented a high peak area of this compound which is described in the literature with a sensory perception of organic, wet earth [38].

It was not possible to associate the compounds Acetaldehyde (**4**) and 2-Methyl-propanal (**8**) through literature to any of these four categories. Dodecene (**34**), described as undesirable in literature, correlates only with sample S as it was the only sample with presence of this compound in negligible trace amount.

Some general dispersion of the VOO samples in between the plot happens because of the differences in their volatile profiles, which are as already mentioned in Chapter 1.2 inherent and dependent of different factors, such as genetic characteristics, geographic origin, pedoclimatic conditions, olive ripening, processing conditions, and olive oil storage [18].

These differences in the volatile profile of the samples are the reason why ultimately it was possible to discriminate between virgin olive oils of different quality and characteristics.

2.5 Conclusion GC-MS analysis

HS-SPME in combination with GC-MS analysis of the 34 different olive oil samples, enabled to identify in average 172 compounds of which in average 51 per sample could be identified through literature research being of sensory relevance and establish an association to their respective sensory attributes.

The GC-MS analysis provided useful information about the presence of major and minor compounds present in the olive oil samples and helps to establish a volatile profile for each analysed sample.

These analysis results are important to evaluate in a next step the extraction efficiency and to determine the success of the applied extraction methods and evaluate the extraction conditions used in order to obtain high quality odour standards that can be used in the training and monitoring of sensory analysis panels. Thereby they lay the foundation for the continuous work in order to obtain through extraction methods the desired odour standards.

The PCA results show the utility of the method as a tool to discriminate between virgin olive oils of different quality. The graphical illustration proved to be a powerful technique for understanding the impact of the various volatile compounds and their concentrations and helps to determine sensory characteristics of virgin olive oils by determining the most influencing ones. It should be noted though, that the high concentration volatile compounds are not necessarily the major contributors of odour [42]. The contribution of each volatile

compound to the whole aroma and flavour is not only related to their concentration in the oil but also to their corresponding sensory threshold [27].

Results revealed though that it was possible to distinguish the sensory defective olive oils from the samples without sensory defect, through their volatile profile.

The following Chapter deals with the practices for the extraction of volatile compounds from food matrix and the applied extraction methods in order to extract and trap the identified volatile compounds from the olive oil samples for further manipulation.

3 Extraction methods

The extraction of volatile compounds is a complex process that is influenced by a variety of factors. Depending on the objective of the extraction, different process techniques and extraction conditions may be used [56, 57].

In this aspect, it is important that extraction conditions are fully optimized with this objective in mind and that these conditions are not universal and may be adjusted for different types of raw materials. The characteristics of the raw material, the solvent used, the extraction temperature and time are the primary variables involved in most extractions and they are irrevocably associated with the success of the process [56, 57].

Generally, large numbers of chemical substances are simultaneously recovered during an extraction process from a natural raw material and it is quite uncommon to find a specific extraction method and a solvent mixture that presents a high and specific selectivity for the main target compounds and that will lead to high purity extracts [56].

However, all these separation/extraction methods must also fulfill most of the established requirements for the extraction methodologies, namely in terms of purification yields, compounds thermal and chemical stabilities, solvents and solvent mixtures (physicochemical properties and potential risks/toxicity), extraction conditions, direct and indirect costs, energy demand, scale-up and processing issues, as well as other important and mandatory environmental or specific legislation issues [56, 58].

Harjo et al. suggested a five-step systematic preliminary evaluation procedure that can be applied for the potential production of phytochemical and other natural-origin products [56, 59]:

(i) specification and characterization of the target compound(s) and natural raw material (conforming to their physicochemical properties);
(ii) selection of the adequate extraction/separation techniques and solvents/solvent mixtures to obtain the target compound(s);
(iii) flow sheet design and selection of required unit operations and equipment;
(iv) selection of the operational conditions for all employed unit operations and equipment; and
(v) flow sheet evaluation modelling, cost information analysis and consideration of other specific criteria which may be relevant for the production and commercialization of the envisaged phytochemical or natural compound.

After the selection and the identification of the envisaged target extracts/compounds and of their potential natural sources, it is then required to define and to select the most efficient methodologies to be employed in the extraction process. This selection is usually directly

related and even limited by the selection of the most suitable solvents and solvent mixtures (in terms of physicochemical properties, purity/composition and potential risks and toxicity) [56, 57, 95].

In general terms, an ‘optimal’ extraction method should preferably: lead to high extraction yields and high purity extracts; employ safe and non-toxic solvents or solvent mixtures; avoid any potential extracts/target compounds degradation or loss; be environmentally and ecologically friendly; meet all required general and specific regulations; be a quick and low-energy consuming method; and be technologically practicable and economically profitable [56, 60].

Nearly all the currently available methods and equipment for the extraction of natural products from solid, and even from liquid raw materials are typically solvent-based processes, thus they need the use of specific solvents or solvent mixtures which are generally in the liquid or in the supercritical state. These extraction methods will involve direct contact between the raw material and the solvent (or solvent mixture) [56, 61].

Increasing consciousness in society with regards to both environmental and human health, has lead to new technological approaches that allow the reduction or (if possible) elimination of solvents from final products. The best strategy for this intention is to completely avoid the use of toxic solvents in every processing step, but in the cases where this is not possible, solvent elimination techniques are necessary [56, 57].

Organic solvents may concentrate in lipid- and fat-rich cells of the human body, as well as in the nervous system, brain, bone marrow, liver, and body fat, and can cause different detrimental effects to health [56].

The success of an extraction method not only depends on the extraction step itself but also on the matrix considered as well as on the analyte trapping system. Quantitative extraction conditions cannot be developed and evaluated unless the collection step is efficient. Therefore, the first task of the analyst is to optimize the collection system and determine its efficiency for the target analytes [62].

For the extraction of the odour compounds in this work, methodologies like distillation and Supercritical fluid extraction were intended to be used, due to availability in the laboratory and compatibility with the works objective. In the following paragraphs a short introduction to the working principles of these techniques is given, referring to similar works.

An in depth literature research about the extraction of volatile compounds from olive oil samples showed that most works done with olive oil aim for the extraction of the VOCs for subsequent analysis in gas chromatography, employing often the use of health hazardous solvents. No similar work with the aim to use these extracts as sensory reference standards were found. Another important fact to bear in mind is the fixing of the volatile aromas onto a carrier material in order to be stable and presentable for sensory panel training.

3.1 Carrier material

Since aromas are volatile, a carrier material is necessary which fixes the aroma in a long term to the desired application site. Furthermore, the carrier also serves to dilute the flavour-intensive aroma and make the dose controllable. The carriers can include among others, ethanol, water, propylene glycol, triacetin or diacetin [63, 64].

These substances are GRAS, colourless and except from ethanol odourless, thus they serve for the defined purpose to be used as reference standard in sensory panel training.

For the training of odour recognition with standardised flavouring substances, odourless brown (colour-masking), sealable glass containers are usually used with odourless paraffin wax or odourless cotton wool, to which the odour solution is then applied so that the carrier substances are saturated with the aromatic substance and these then evaporate into the gas headspace of the sealed glass vessel. A further possibility is the uptake of the aromatic substance via smelling strips or capsules as well as sniffing sticks that contain the odour substance for the odour test [8].

3.2 Supercritical fluid extraction (SFE)

Supercritical fluid extraction (SFE), mainly using carbon dioxide is an available and promising process to extract volatile compounds from natural products. It is especially interesting, because in comparison to the traditionally used solvent extraction processes it avoids the thermal degradation of aroma compounds and the presence of solvent residues in the extracts [65].

In supercritical fluid extraction one component (the extractant) is seperated from another (the matrix) using supercritical fluids as the extracting solvent [66].

Carbon dioxide is often the preferred solvent in the food industry because it is non-toxic, non-corrosive, low-cost, non-flammable, readily available and has low critical temperature and pressure [65].

The system contains a pump for the CO_2, a pressure cell to contain the sample and means of maintaining pressure in the system and a collecting vessel. The liquid is pumped to a heating zone, where it is heated to supercritical conditions. It then passes into the extraction vessel, where it rapidly diffuses into the solid matrix and dissolves the material to be extracted. The dissolved material is swept from the extraction cell into a separator at lower pressure, and the extracted material settles out. The CO_2 can then be cooled, recompressed and recycled, or discharged to atmosphere [66].

A schematic diagram of the supercritical fluid extraction system is illustrated in Figure 8.

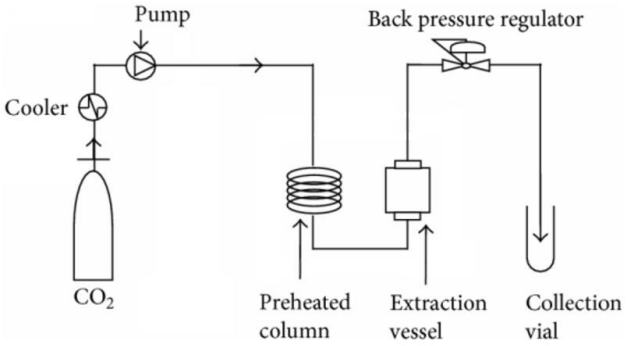


Figure 8: Schematic diagram of supercritical fluid extraction system.

Two different approaches are commonly used for trapping of the target analytes: liquid solvent collection and solid-phase trapping. Both systems have their advantages and disadvantages regarding the ease of handling, choice of restrictor type, maximum gas flow, and compatibility with the various types of supercritical fluids, modifiers and analytes [62].

Many studies involving the extraction of natural aroma compounds by this procedure, for example for rosemary, vanilla, coriander seeds, black pepper or peppermint, etc. are reported [65].

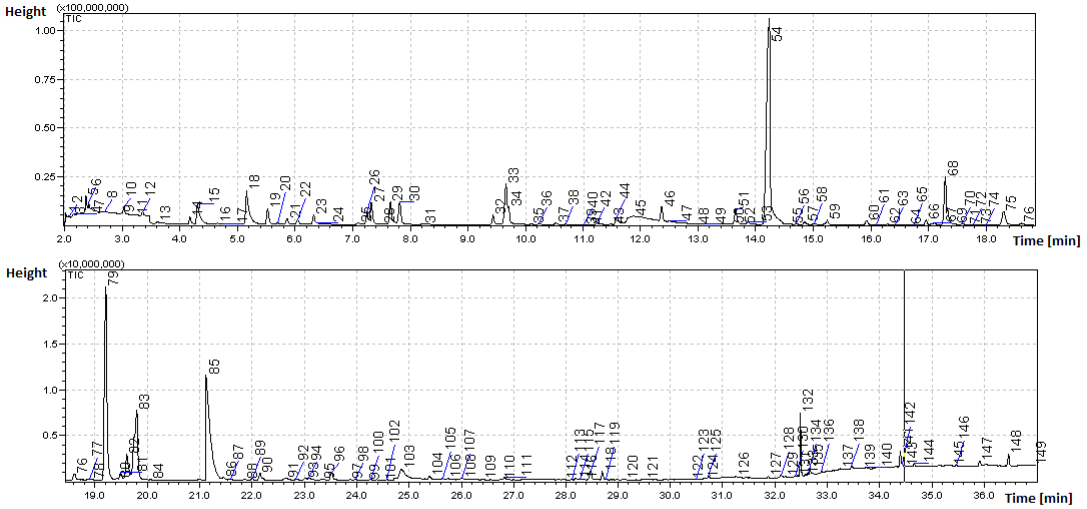
3.2.1 Equipment and materials

The following equipment and materials were used for the olive oil extraction with SFE:

Table 10: SFE sample extraction: equipment and materials.

Supercritical fluid extraction system	SFE: Thar Technology, Pittsburgh, PA, USA, model SFE-500F-2-C50, comprising a 500 mL cylinder extraction cell; CO_2 pump: Thar SFC P-50 high pressure pump; Automated back pressure regulator: TharSFC ABPR, Thar Technology, Pittsburgh, PA, USA
Olive oil support material	Silica gel: Scharlau - Silica gel 60, 0,04 – 0,06 mm, for flash chromatography (230 – 400 mesh ASTM)
	Sepiolite: Aldrich Chemistry – Sepiolite powder – Concentration: ~13% Mg
	Charcoal: Ceca Acticarbone S n°23270
	Flour: Generic wheat flour from supermarket
	Sea Sand for analysis: PanReac Applichem ITW Reagents – Particle size 0,3 mm
Collecting solvent	20% ethanol
	Propylene glycol: Fagron – Propylenglycolum (76,1) $C_3H_8O_2$ d. = 1,038
	Refined oil: Fula - Natural-scented food oil. 100% vegetable. Refined sunflower oil, refined sunflower oil high in oleic acid, refined corn oil, natural aroma

For the extraction attempts EVOO sample “X” from Table 8 in Chapter 2.4 was used, for the simple reason of having the largest amount of all samples at disposal for use. Its chromatographic profile and volatile compounds identified are shown in Figure 9.



Number	Cumpound	RT	Area %	Similarity %
1	Hydrazinecarboxamide	2.027	0.15	
2	Pentane (CAS) n-Pentane	2.078	0.06	
3	1,4-Dioxane-2,6-dione	2.133	0.01	
4	2-Methyl-1-butene	2.153	0.13	
5	1,3-Pentadiene, (E)-	2.370	0.76	
6	1,3-Pentadiene, (E)-	2.422	0.23	
7 ☆	Acetaldehyde (CAS) Ethanal	2.468	0.04	98
8	Borane-methyl sulfide complex	2.702	0.01	
9 ☆	Propanal (CAS) Propionaldehyde	2.991	0.02	92
10 ☆	Octane	3.024	0.32	96
11	2-Propanone (CAS) Acetone	3.214	0.01	
12 ☆	Acetic acid, methyl ester (CAS) Methyl acetate	3.355	0.26	97
13	Cyclotrisiloxane, hexamethyl-	3.608	0.22	
14 ☆	ETHYL ACETATE	4.176	0.50	98
15	Methanol (CAS) Carbinol	4.303	3.02	
16 ☆	Butanal, 2-methyl-	4.653	0.06	95
17 ☆	Butanal, 3-methyl- (CAS) 3-Methylbutanal	4.758	0.03	95
18 ☆	Ethanol	5.157	5.60	97
19	Hexane, 1-methoxy-	5.521	1.54	
20	Furan, 2-ethyl-	5.692	0.08	
21	3-Ethyl-1,5-octadiene	5.863	0.53	
22	3-Ethyl-1,5-octadiene	6.045	0.47	
23 ☆	3-Pentanone (CAS) Diethyl ketone	6.322	0.66	95
24 ☆	PENTANAL	6.375	0.00	87
25	Cyclotetrasiloxane, octamethyl-	7.095	0.12	
26	3-Hexene, 1-methoxy-, (Z)-	7.246	0.79	
27	3-Ethyl-1,5-octadiene (c,t)	7.322	1.68	
28	.alpha.-pipene	7.510	0.04	
29 ☆	1-Penten-3-one (CAS) Ethyl vinyl ketone	7.648	2.37	97
30	3-Ethyl-1,5-octadiene (c,t)	7.811	2.08	
31 ☆	Benzene, methyl- (CAS) Toluene	8.213	0.08	96
32	3-Ethyl-1,5-octadiene (c,t)	9.434	0.80	
33 ☆	Hexanal	9.655	2.75	
34	3-Ethyl-1,5-octadiene (c,t)	9.697	0.05	94
35	7-Octen-2-ol	10.096	0.01	
36 ☆	1-Propanol, 2-methyl- (CAS) Isobutyl alcohol	10.231	0.03	96
37 ☆	2-Pentenal, (E)-	10.516	0.17	94
38	3-Pentanol	10.691	0.02	
39 ☆	Benzene, ethyl- (CAS) EB	11.005	0.01	91
40	1-Butanol, 3-methyl-, acetate (CAS) Isoamyl acetate	11.025	0.00	
41	2-Butanol, 3-methyl-	11.095	0.01	
42 ☆	2-Pentenal, (E)-	11.255	0.33	93
43	1,2-DIMETHYLBENZENE	11.491	0.03	
44 ☆	4-Pentenal, 2-methyl-	11.590	0.96	92
45 ☆	CIS-3-HEXENAL	11.868	4.49	94
46 ☆	1-Penten-3-ol	12.371	1.31	98
47	Cyclopentasiloxane, decamethyl-	12.502	0.11	
48	o-Xylene	12.968	0.01	
49 ☆	Heptanal	13.111	0.03	94
50	2-Butenal, 3-methyl-	13.568	0.01	
51 ☆	2-Hexenal, (E)-	13.654	1.55	93
52	2-Hexenal, (E)-	13.760	0.00	83
53 ☆	1-Butanol, 3-methyl- (impure) (CAS) 3-Methyl-1-butanol	13.869	0.22	96
54 ☆	2-Hexenal, (E)-	14.239	38.66	90
55	Furan, 2-pentyl-	14.603	0.00	
56	1,3,6-Octatriene, 3,7-dimethyl-, (E)-	14.707	0.01	
57	5-Octadecene, (E)-	14.854	0.26	
58	1-Dodecanol, 3,7,11-trimethyl-	15.010	0.02	
59	1,3,6-Octatriene, 3,7-dimethyl-, (Z)-	15.240	0.45	
60	Acetic acid, hexyl ester	15.927	0.38	
61 ☆	Benzene, 1,2,4-trimethyl- (CAS) 1,2,4-Trimethylbenzene	16.099	0.01	90
62	2-Butanone, 3-hydroxy- (CAS) Acetoin	16.286	0.04	
63 ☆	Octanal	16.414	0.02	96
64		16.641	0.01	
65	2-Propanone, 1-hydroxy- (CAS) Acetol	16.745	0.01	
66	1,5-Heptadiene, 3,3-dimethyl-, (E)-	16.956	0.42	
67	Cyclopentanol	17.092	0.15	
68	3-Hexen-1-ol, acetate, (Z)-	17.286	5.97	
69	2-Heptenal, (Z)- (CAS) CIS-HEPT-2-ENAL	17.447	0.00	
70	1,2-Bis(.gamma.-trimethylsilypropoxy)ethane	17.588	0.07	
71	(E)-7-methyl-1,6-dioxaspiro[4.5]decane	17.671	0.01	
72		17.776	0.00	
73 ☆	5-Hepten-2-one, 6-methyl-	17.867	0.02	94
74		18.006	0.00	
75 ☆	1-Hexanol	18.301	2.00	97
76 ☆	3-Hexen-1-ol, (E)-	18.614	0.17	97
77		18.900	0.00	
78	Acetonitrile, dichloro-	18.931	0.00	
79 ☆	3-Hexen-1-ol, (Z)-	19.209	5.30	97
80	Benzene, (methoxymethyl)- (CAS) Benzyl methyl ether	19.427	0.02	
81 ☆	Nonanal	19.493	0.13	95
82 ☆	2,4-HEXADIENAL 2	19.609	0.57	96
83 ☆	2-Hexen-1-ol, (Z)-	19.798	2.44	93
84 ☆	2-Hexen-1-ol, (Z)- (CAS) CIS-HEX-2-EN-1-OL	20.066	0.00	85
85 ☆	Acetic acid (CAS) Ethylic acid	21.128	4.73	98
86 ☆	2,4 HEPTADIENAL	21.452	0.03	95
87 ☆	2-Butanone (CAS) Methyl ethyl ketone	21.596	0.01	83
88	(+)-Cycloisosativene	21.857	0.02	
89	Trisiloxane, 1,1,1,5,5,5-hexamethyl-3,3-bis[(trimethylsilyl)oxy]-	22.008	0.04	
90 ☆	.alpha.-Copaene	22.154	0.18	95
91		22.644	0.09	
92	2,3-DIMETHYL-4-HYDROXY-2-BUTENOIC LACTONE	22.844	0.03	
93 ☆	Benzaldehyde	23.019	0.03	97
94		23.090	0.01	
95		23.332	0.01	
96 ☆	3-Pentanone	23.515	0.19	86
97	1-Octanol	23.875	0.02	
98	Dimethyl Sulfoxide	23.974	0.02	
99		24.183	0.00	
100		24.260	0.01	
100		24.260	0.01	
101	Propanoic acid, 2,2-dimethyl- (CAS) Pivalic acid	24.488	0.00	
102	Bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-6-(4-methyl-3-pentenyl)-	24.583	0.01	
103	2(5H)-Furanone, 5-ethyl-	24.852	0.46	
104	Ethanol, 2-(2-ethoxyethoxy)-	25.390	0.06	
105	Butyrolactone	25.637	0.02	
106 ☆	Butanoic acid	25.742	0.01	94
107	SILICATE ANION TETRAMER	26.001	0.01	
108		26.025	-0.00	
109 ☆	1-Nonanol	26.408	0.01	90
110		26.768	0.01	
111		26.823	0.03	
112	.alpha.-Muurolene	27.952	0.02	
113		28.104	0.01	
114	1,4-Cyclohex-2-enedione	28.201	0.02	
115		28.285	0.00	
116 ☆	Pentanoic acid	28.331	0.01	84
117 ☆	.alpha.-Farnesene	28.485	0.18	96
118	1,5-Heptadien-4-one, 3,3,6-trimethyl-	28.707	0.15	
119	2,4-Hexadienoic acid, (E,E)- (CAS) Sorbistat	28.778	0.01	
120	Methyl Salicylate	29.136	0.02	
121	SILICATE ANION TETRAMER	29.532	0.01	
122		30.381	0.01	
123	1,4-Butanediol (CAS) Sucol B	30.492	0.01	
124	Propylene Carbonate	30.662	0.01	
125 ☆	Hexanoic acid (CAS) n-Hexanoic acid	30.732	0.05	94
126	Benzyl Alcohol	31.258	0.02	
127	Phenylethyl Alcohol	31.871	0.03	
128	1,4-Butanediol	32.118	0.05	
129	SILICATE ANION TETRAMER	32.200	0.01	
130	Hexanoic acid, 2-ethyl-	32.380	0.01	
131 ☆	Heptanoic acid	32.427	0.01	81
132	Pentanoic acid, 3-methyl-2-oxo-, methyl ester	32.490	0.69	
133	Ethanol, 2,2'-oxybis- (CAS) Diethylene glycol	32.587	0.01	
134	3-Hexenoic acid, (E)-	32.627	0.02	
135	3-Hexenoic acid	32.669	0.07	
136	Phenol (CAS) Izal	32.892	0.01	
137	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, (E)-	33.239	0.01	
138 ☆	Octanoic Acid	33.461	0.04	90
139		33.659	0.01	
140	1-Octadecanol (CAS) Stenol	33.743	0.03	
141	Nonanoic acid	34.395	0.18	
142		34.432	0.00	
143		34.467	-0.00	
144	Ethanol, 2-[2-(2-butoxyethoxy)ethoxy]-	34.658	0.04	
145	Decanoic acid (CAS) Capric acid	35.366	0.01	
146		35.457	0.01	
147		35.912	0.09	
148	Diethyl Phthalate	36.459	0.18	
149		36.935	0.01	

Figure 9: GC-MS analysis results of the EVOO sample “X”, used for extraction purposes in this Chapter. (Marked with star, compounds identified of sensory relevance).

3.2.2 Sample preparation and analysis conditions

Morales et al. applied Supercritical fluid extraction (SFE) for the extraction of volatile compounds of virgin olive oil and olive fruit samples.

In order to collect the volatile components of these samples, the built-in extract trap of the SFE system was bypassed. Instead, the SFE extract, together with the total volume of venting carbon dioxide was purged through a removable Tenax TA trap. The traps were subsequently desorbed onto a GC column by thermal desorption for volatile compound analysis [1, 67]. Different profiles of volatile compounds, from flavours to off-flavours were obtained by Morales et al. by changing SFE operational parameters. Softer extraction conditions were applied to obtain volatile compounds more representative of the virgin olive oil flavour, while more drastic conditions (higher temperature and pressure) achieved a volatile compound profile with more presence of off-flavours, by oxidising the olive oil samples [67].

The initial optimum extraction conditions for the extraction of volatile compounds from olive oil were oriented based on Morales et al. findings, as shown in Table 11 [67].

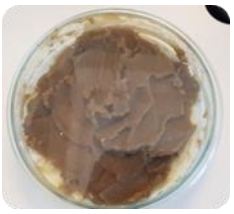
Table 11: Optimum extraction conditions of volatile compounds from olive oil matrix, used by Morales et al. [67].

SFE extraction conditions	
Extracting solvent	CO_2
Density (g/ml)	0.25
Pressure (bar)	81
Temperature (°C)	45
Static time (min)	1
Dynamic time (min)	30
Flow-rate (ml/min)	1
Oil/silica ratio	4.2/50
Co-solvent	-
Collecting adsorbent	Tenax TA Trap

As olive oil is a liquid matrix, it is necessary to use a support material for the extraction process, in order to solidify the matrix. Morales et al. evaluated two different support materials. Filter paper and silica gel. Filter paper was considered as inadequate, as leaks were detected during trials. In many cases adsorbing the samples onto solid-phase sorbents can also enhance the class selectivity or class fractionation of the performing extraction [67].

During this work more support materials were tested for extraction purpose. Besides silica gel, sepiolite, charcoal, flour and sea sand for analysis were employed. The latter one was excluded for extraction, as the mixture liquefied already again at ambient temperature. Olive oil samples were mixed as listed in Table 12 in the following ratios with a spatula:

Table 12: Support material/olive oil ratio for supercritical fluid extraction.

Support material	Ratio SM/OO	Cost benefit to retain 5g olive oil	
Sepiolite	1g/2,89g	≈ 0,10€	
Silica gel	1g/1,5g	≈ 0,31€	
Charcoal	1g/1,93g	≈ 2,66€	
Flour	1g/0,43g	≈ 0,0052€	
Sea sand for analysis	1g/0,28g	≈ 0,95€	

For each extraction attempt, ten grams of the mixture of support material and olive oil were introduced into the extraction cell of the SFE apparatus. Void space was filled with glass beads. Liquid solvent collection was used for the collection of the VOCs as it is mechanically simple, economical and has been the most widely used approach for natural samples. In this approach the end of the SFE flow restrictor is placed directly into the collection solvent, and the CO_2–analyte mixture is depressurized directly in contact with the solvent/into the collection liquid, as illustrated in Figure 10.

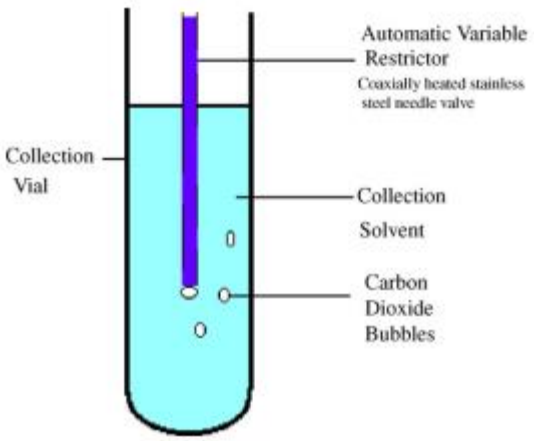


Figure 10: Schematic representation of the liquid trapping process involving immersion of the restrictor into a liquid solvent [62].

Different collecting solvents as 20% ethanol, propylene glycol and refined oil, as well as different extraction conditions were tested for extracting and trapping the volatile compounds. All extracts after the extraction process were analysed by GC-MS under the same analysis conditions as used for the olive oil samples in Chapter 2.2. During the extraction, the collection vial was kept in ice and afterwards stored in the refrigerator until analysis by GC-MS. During all extraction attempts CO_2 flow-rate was kept constantly at 5 ml/min as it was the lowest flow rate possible, restricted by equipment capacity.

Applied extraction conditions and attempts with observations are shown in Table 13.

Table 13: SFE of olive oil sample, extraction conditions and attempts (marked in red, conditions differing from pre-defined conditions from Morales et al. [67]).

Extraction conditions	Density (g/ml)	Pressure (bar)	Temp. (°C)	Static time (min)	Dynamic time (min)	Flow-rate (ml/min)	Oil/silica ratio	Co-Solvent	Collecting adsorbent	Observation
Pre-defined (Literature)	0.25	81	45	1	30	1	4.2/50	-	Tenax TA Trap	-
1. Extr	0.25	80	45	2	60	5	6g Oil/4g Silica gel	-	15 ml Propylene glycol	No olive oil smell
2. Extr	0.25	80	45	2	60	5	7,4g Oil/2,6g Sepiolite	-	15 ml Propylene glycol	No olive oil smell
3. Extr.	0.81	200	45	2	60	5	7,4g Oil/2,6g Sepiolite	-	15 ml Propylene glycol	No olive oil smell, fat got extracted by high pressure
4. Extr.	0.51	100	45	2	30	5	7,4g Oil/2,6g Sepiolite	-	35 ml 20% Ethanol	No olive oil smell
5. Extr.	0.41	80	37	2	30	5	6,6g Oil/3,425g Charcoal	-	35 ml 20% Ethanol	Slight smell like green olives
6. Extr.	0.41	80	37	2	30	5	6,6g Oil/3,425g Charcoal	-	25 ml Propylene glycol	No olive oil smell
7. Extr.	0.41	80	37	2	30	5	6g Oil/4g Silica gel	10% Ethanol	25 ml 20% Ethanol	Slight smell like olive oil/olives, but the ethanol disturbs a lot
8. Extr.	0.41	80	37	2	30	5	6g Oil/4g Silica gel	10% Ethanol	25 ml Propylene glycol	No olive oil smell
9. Extr.	0.41	80	37	3:30	30	5	3g Oil/7g flour	-	25 ml 20% Ethanol	No olive oil smell
10. Extr.	0.70	80	30	2	30	5	6,6g Oil/3,425g Charcoal	-	20 ml Refined oil	No smell at all
11. Extr.	0.41	80	37	2	30	5	6,6g Oil/3,425g Charcoal	-	25 ml Refined oil	No olive oil smell, but slightly chemical smell
12. Extr.	0.70	80	30	2	30	5	6,6g Oil/3,425g Charcoal	-	40 ml 20% Ethanol	No olive oil smell

3.2.3 Results and discussion

SFE results in Table 13 show that by smelling on the extracts directly after extraction only in extraction attempt number five and seven it was observed a slight smell reminiscent of olives or olive oil, although the smell of ethanol influenced a lot in the odour sensation.

However, the two in no way resembled the smell of the olive oil sample that had been extracted and did not suit to be used as sensory reference standards. After opening the extracted samples again for analysis in the GC, the samples no longer presented an olive oil or olive-related smell, what could be the consequence of volatilization of the compounds or a diminution of concentration.

As the predefined extraction conditions showed no result as by odour recognition, the extraction conditions were altered. Too high pressure (third extraction with 200bar) resulted in fat particles in the extract. Extraction under subcritical conditions (temperature under 37°C, extraction number ten and twelve) did also not result in extracts with odour recognition. Neither did using refined oil as collecting solvent.

Extractions number five and six were done under exactly the same extraction conditions, only changing the collecting solvent from ethanol to propylene glycol.

The same was done in extraction number seven and eight, using silica gel as support material for the olive oil and 10% ethanol as co-solvent to increase CO_2 polarity for the extraction.

Observations showed, that even though the extraction conditions were the same, only by using 20% ethanol as collecting solvent it was possible to resemble slightly an olive or olive oil reminiscent odour, on the contrary to propylene glycol.

This could be attributable to the fact that water possesses a higher polarity than propylene glycol according to the dialectric constant which is the index of a compounds polarity (see Table 14) [68]. As According to Flath, Forrey and Guadagni the contribution of the non-polar fraction to the olive oil aroma is considered minimal, it could be therefore possible that the water binding force of the polar compounds is higher [24]. As polar molecules are more soluble in polar solvents (like dissolves like) [95].

Table 14: Dielectric constant of water, ethanol and propylene glycol and refined sunflower oil at 20°C. (The higher, the more polar) [68, 69].

Compound	Dielectric constant δ, at 20°C
Water	80
Ethanol	25
Propylene glycol	32.1
Refined sunflower oil	22.5

As the first four extraction attempts didn't show any results from an odour perspective of the extracts, using silica gel and sepiolite as support matrix, it was taken into consideration if either there was an problem with the low polarity of the CO_2 or if it should be tried to use support materials for the olive oil matrix that have a weaker polarity. Many literature reviews report about the drawbacks of CO_2 as solvent for SFE because of its low polarity. Many non-polar to moderately polar compounds can be extracted with carbon dioxide, while more polar compounds can be extracted with other fluids or modified carbon dioxide [62, 70].

Several options were taken into consideration to overcome this drawback:

1. Adding ethanol as a modifier to CO_2 to increase the solvent polarity [71, 72].

2. Testing charcoal as support material to solidify the olive oil matrix, instead of silica gel or sepiolite, which are polar and might bind the polar volatile compounds, interfering in extraction. As charcoal is non-polar and therefore should not retain so much the volatile olive compounds.

3. Using SPE cartridges or discs as support material which are used in combination with liquid matrices in SFE, inside the SFE extraction cell. Apparently these SPE discs often need to be activated first with methanol, in this case, they probably no longer fit for this purpose, as methanol is toxic. In literature these discs are reported in relationship with aqueous matrices, not in connection with oil or olive oil [73].

Figure 11: SPE extraction disks usable for extraction of liquid matrices in SFE [74].

4. Freeze-drying to solidify the matrix [75]. But as olive oil has practically no water, it was considered another option to dispense.

5. In situ derivatization in the extraction cell that serves to lower the polarity of the extracted matrix and increase its volatility. Through the addition of derivatization reagents to the extraction cell, e.g. trymethylchlorosilane (TMCS) and hexamethyldisilizane (HMDS) [76].

6. Simply mixing the olive oil with some commercial flour, a starch or an odourless powder, just to solidify the olive oil matrix and put it into the extraction cell.

Easy to implement and viable only seemed to be the first three options and option number six, adding ethanol as modifier to CO_2, testing charcoal and/or flour as support matrix for the olive oil. However, no extraction with SPE cartridges or disk was tested.

After applying the mentioned options in extraction attempt number five in Table 13, using charcoal as support material, it was possible to attain results in as a slight smell like green olives. Applying 10% ethanol as modifier in extraction attempt number seven, using silica gel as support material resulted in slight smell like olive oil/olives, although the ethanol smell disturbed a lot. In both cases the smell after a period of storage had already faded away completely.

3.2.3.1 GC-MS analysis results of extracts

Chromatographic results of the extracts analysed by GC-MS were grouped together for better illustration purpose by the collecting solvent used, as it had the most influence on the extracts chromatographic profile and thus their volatile compounds profile, as can be seen in the following chapters.

3.2.3.1.1 Propylene glycol extracts

As it is possible to see in Figure 12 by overlapping the extraction chromatograms used with the collecting solvent propylene glycol with the propylene glycol standard chromatogram and a chromatographic profile of the extracted olive oil sample, the extracts chromatographic profile resemble mostly the peaks appearing in the chromatographic profile of the propylene glycol standard.

Exceptions are besides some peaks appearing with compounds with no sensory relevance, a large peak at minute five from **ethanol** and minor peaks of sensory relevance, which are marked in Figure 12. The **ethanol** peak might appear because of cleaning residues of the equipment used, as the equipment of SFE and its system is cleaned using ethanol.

Extraction attempt number eight shows a higher peak in ethanol because in this attempt it was used 10% ethanol as a modifier for extraction, in purpose to increase the CO_2 polarity of SFE.

A spot test of the average spectrum for the large peak extending from approximately minute 24.50 to minute 25.50 at the beginning of the peak, confirmed its characteristical affiliation to the compound propylene glycol with a similarity of 96 percent, practical kovats showed to be identical with the theoretical ones found in literature.

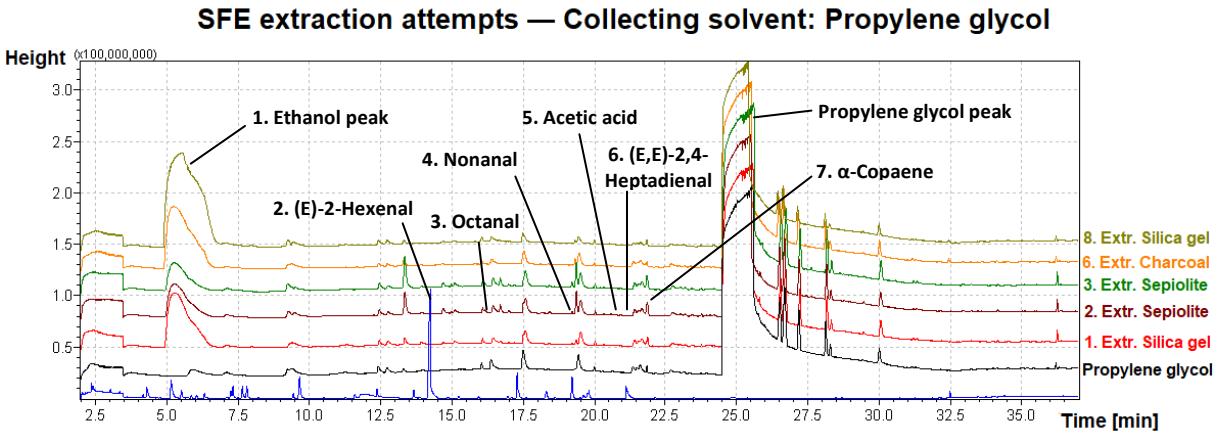


Figure 12: GC-MS analysis results of SFE extracts of olive oil sample, using propylene glycol as collecting solvent.

A fragmentation profile was done for the ethanol peak at minute five, for the extract number one. In order to confirm the eventual presence of compounds extracted from the olive oil sample inside this peak area, by identifying the presence of the compounds mass spectral fragments in the ethanol peak area (see Figure 13).

Results confirmed the presence of the fragments 31 and 32 and the compound ethanol, as well as the fragment 45 from 1-Methoxyhexane and fragments 39 and 43 from 3-Pentanone in the peak, but the presence of the fragment 55 from 3-Ethyl-1,5-octadiene in the peak was not confirmed, as well not the fragments 67 and 81 from 2-Ethylfuran.

Spot tests of the average spectrum in the beginning, the middle and at the end of the ethanol peak confirmed ethanol at the beginning and the end of the peak, but showed Methylhydrazine with 86% similarity and Formic acid with 81% similarity for the middle of the peak.

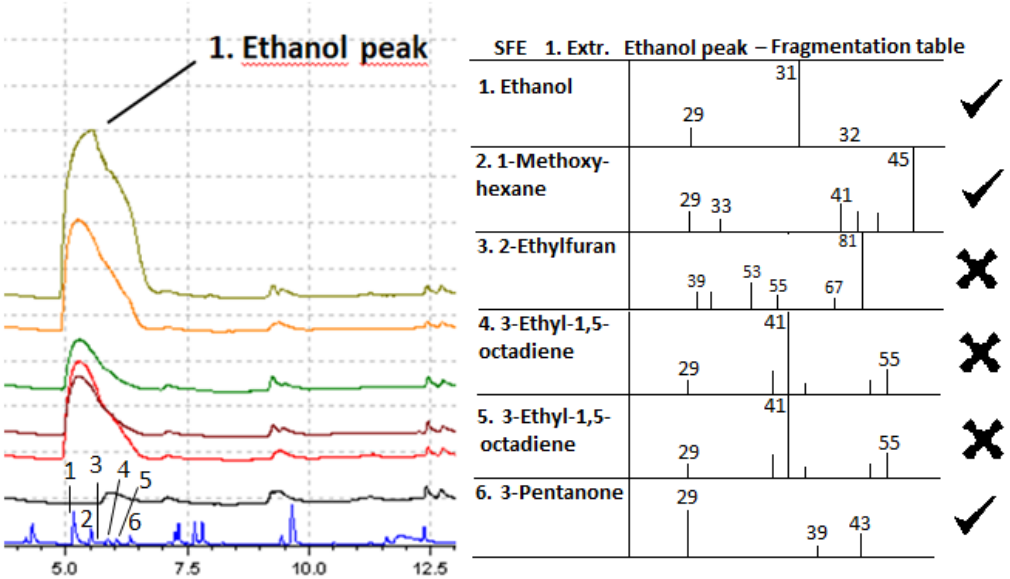
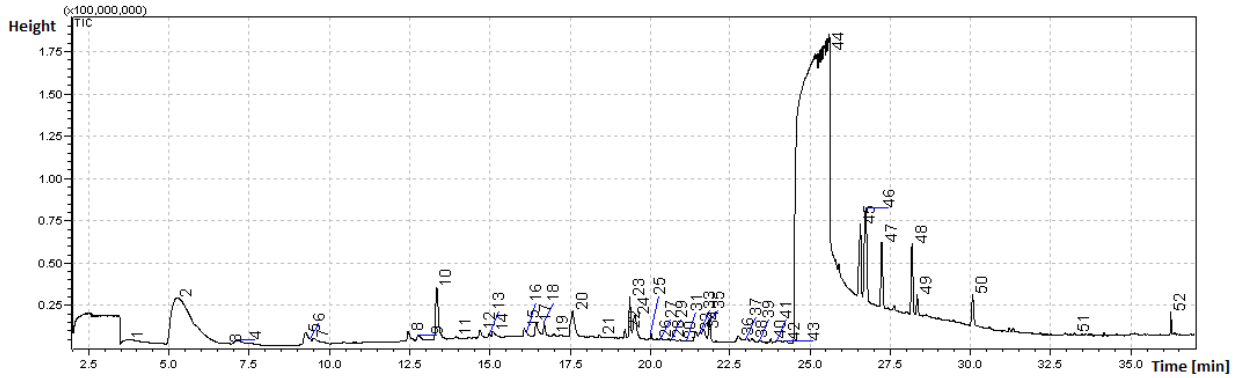


Figure 13: Fragmentation table for ethanol peak in extract number one, on the right side mass spectrums of the compounds.

All peaks appearing in the extract number three are identified and listed below for illustration in Figure 14. Compounds marked with a star, are of sensory relevance and also appear in the olive oil sample.



Number	Compound	RT	Area %	Similarity %
1		3.750	0.66	
2 ☆	Ethanol (CAS) Ethyl alcohol	5.258	10.33	91
3	Decane	6.798	0.04	
4	Cyclotetrasiloxane, octamethyl-	7.122	0.25	
5		9.248	0.28	
6	Cyclopropane, 1,1-dimethyl-2-(2-propenyl)-	9.388	0.01	
7		9.502	0.06	
8	Cyclopentasiloxane, decamethyl-	12.451	0.31	
9		12.766	0.22	
10	Tridecane	13.341	1.61	
11 ☆	(E)-2-Hexenal	13.933	0.06	91
12	5-Octadecene, (E)- (CAS)	14.685	0.17	
13	1,3,6-Octatriene, 3,7-dimethyl-, (Z)-	14.976	0.05	
14	Styrene	15.092	0.13	
15	Cyclotetrasiloxane, octamethyl- (CAS) 1,1,3,3,5,5,7,7-OCTAMETHYL-CYCLOOCTASILOXANE	16.070	0.08	
16 ☆	OCTANAL	16.135	0.02	96
17	2-Propanone, 1-hydroxy- (CAS) Acetol	16.437	0.40	
18	1,5-Heptadiene, 3,3-dimethyl-, (E)-	16.690	0.22	
19	CIS-3-HEXENYL ACETATE	16.983	0.04	
20	2-Propanol, 1-(2-propenyloxy)- (CAS) 1-ALLYLOXY-2-PROPANOL	17.570	1.02	
21	3,5-Dimethyldodecane	18.410	0.04	
22 ☆	Nonanal (CAS) n-Nonanal	19.203	0.13	95
23	Tridecane	19.357	0.58	
24	5-Hexen-2-ol (CAS) 1-Hexen-5-ol	19.506	0.58	
25		20.015	0.08	
26	2-Octenal, (E)-	20.198	0.02	
27	Octanoic acid, ethyl ester	20.354	0.02	
28	3-Tetradecene, (Z)-	20.594	0.03	
29 ☆	Acetic acid (CAS) Ethylic acid	20.701	0.02	85
30	.alpha.-Cubebene	20.949	0.02	
31 ☆	TRANS,TRANS-2,4-HEPTADIENAL	21.165	0.01	89
32	Dipropylene glycol monomethyl ether	21.436	0.17	
33		21.574	0.06	
34	Dipropylene glycol monomethyl ether	21.686	0.15	
35 ☆	alpha-Copaene	21.862	0.40	93
36	2-Propanol, 1-(2-methoxypropoxy)-	22.768	0.17	
37	Decane, 1-chloro-	23.029	0.05	
38		23.202	0.06	
39	1-Propanol, 2-(2-methoxy-1-methylethoxy)- (CAS) 2-(2-METHOXY-1-METHYLETHOXY)-1-PROPANOL	23.436	0.07	
40		23.776	0.04	
41		23.951	0.01	
42		24.210	0.01	
43	Bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-6-(4-methyl-3-pentenyl)-	24.263	0.01	
44		25.608	73.74	
45	2-Propanol, 1-[1-methyl-2-(2-propenyloxy)ethoxy]- (CAS) 1-(2-ALLYLOXY-1-METHYLETHOXY)-2-PROPANOL	26.573	1.65	
46	Butanoic acid, 3-oxo-, 1,1-dimethylethyl ester	26.738	2.42	
47	PROPYLENE GLYCOL TRIMER 4	27.238	1.26	
48		28.178	1.13	
49	PROPYLENE GLYCOL TRIMER 6	28.340	0.28	
50	2-Propanol, 1,1'-oxybis-	30.070	0.60	
51 ☆	Octanoic Acid	33.231	0.02	94
52	Diethyl Phthalate	36.243	0.21	

Figure 14: GC-MS analysis results of SFE extraction attempt number three, using propylene glycol as collecting solvent (marked with star, compounds of sensory relevance also appearing in olive oil sample).

Table 15 gives a general overview of the extraction results of sensory relevant compounds and extraction conditions used, using propylene glycol as collecting solvent.

The subsequent following Figure 15 shows a graphical comparison of the extraction results illustrated in Table 15.

Table 15: Overview of extraction results and extraction conditions using propylene glycol as collecting solvent, peak areas in percent (marked in red, peak areas standing out).

	Peak areas %									
Compounds	**Olive oil**	**Propyle-ne glycol**	**Silica gel**	**Sepioli-te**	**Char-coal**	**1. Extr. Silica gel**	**2. Extr. Sepiolite**	**3. Extr. Sepiolite**	**6. Extr. Charcoal**	**8. Extr. Silica gel**
(E)-2-Hexenal	38,66	-	-	-	-	0,10	0,04	0,06	-	-
Octanal	0,02	-	-	-	-	-	0,02	0,02	-	-
Nonanal	0,13	-	0,04	0,09	-	0,06	0,06	0,13	0,01	0,00
Acetic acid	4,73	0,03	0,15	0,18	-	0,01	0,01	0,02	0,01	0,01
(E,E)-2,4-Heptadienal	0,03	-	-	-	-	-	0,00	0,01	-	-
alpha-Copaene	0,18	-	-	-	-	-	-	0,40	0,01	0,00
Octanoic Acid	0,04	-	-	-	-	0,01	0,02	0,02	0,01	-
Extraction conditions						45°C, 80 bar, 60min static, 2min dynamic, silica gel, 15ml PG	45°C, 80 bar, 60min static, 2min dynamic, sepiolite, 15ml PG	45°C, 200 bar, 60min static, 2min dynamic, sepiolite, 15ml PG	37°C, 80 bar, 30min static, 2min dynamic, charcoal, 15ml PG	37°C, 80 bar, 30min static, 2min dynamic, silica gel, 15ml PG

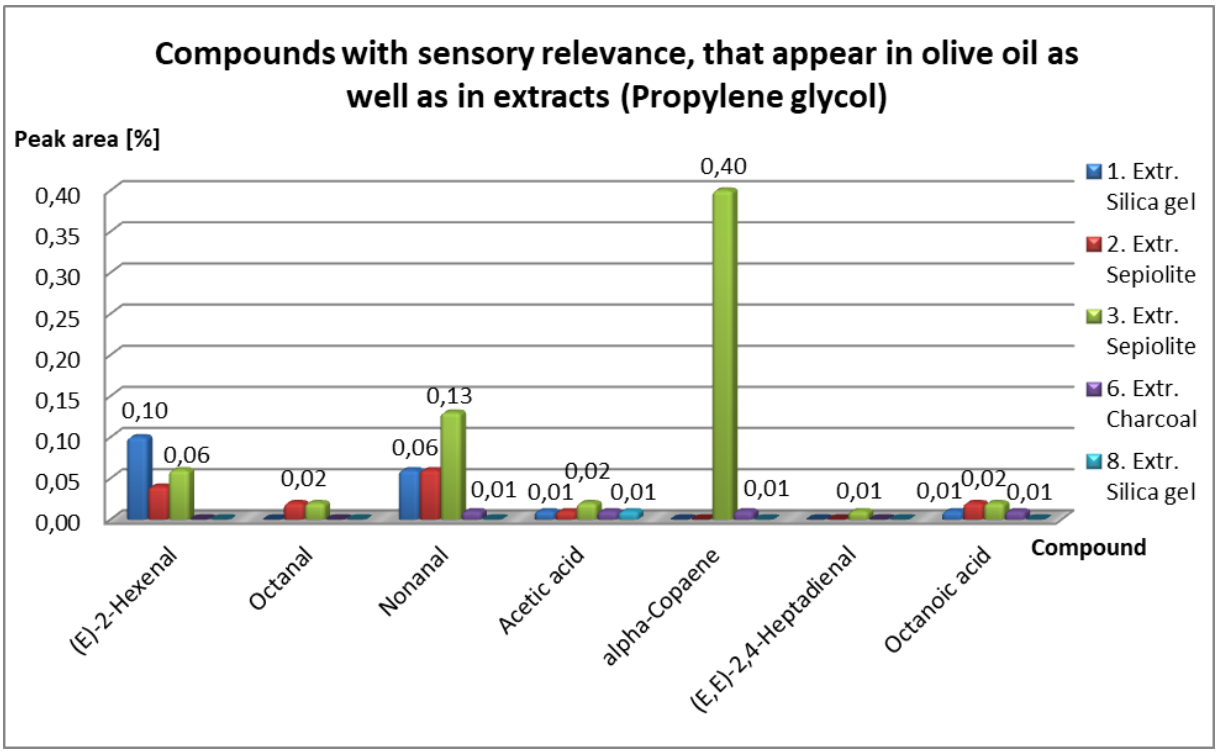


Figure 15: Graphical comparison of extraction results of SFE extraction, using propylene glycol as collecting solvent.

Looking at the peak area results shown in Table 15 and in Figure 15 it is visible that the extraction or collecting results of the olive oil compounds of sensory relevance from the olive oil sample in general were poor. Seven different compounds of sensory relevance were identified in the extracts, out of 49 identified in the for the extraction used olive oil sample, using propylene glycol as collecting solvent.

Octanal, Nonanal, (E,E)-2,4-Heptadienal and Octanoic acid are compounds related to oxidation and rancidity, whose odours are described in the literature as fatty or rancid.

Out of the five SFE extraction attempts using propylene glycol as collecting solvent, extraction attempt number three stands out with an extraordinary large peak of **alpha-Copaene**, but also larger peaks in the other trapped compounds.

This has to do with the higher pressure applied in this extraction attempt, applying 200bar, instead of 80bar in the other attempts.

It seems like a high pressure induces a higher emission or amount of the volatile compound alpha-Copaene. No studies explaining the relationship between alpha-Copaene and a higher pressure were found. Alpha-Copaene is a compound found in a number of essential oil-producing plants [77]. Its odour in olive oil is described as sweet, fruity [78, 79].

The chemical structure of this compound is depicted in Figure 16 [80].

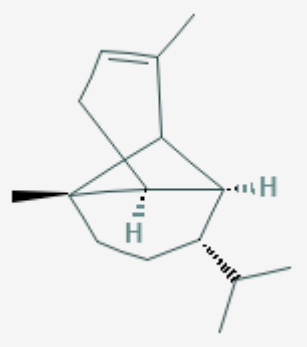


Figure 16: Chemical structure of alpha-Copaene.

Nonanal is absent or present only in traces in fresh virgin olive oils of good quality and shows a positive relationship with oxidation. Angerosa proposed to monitor the oxidation stages of a virgin olive oil during an accelerated thermal oxidation process through the determination of the concentration of Nonanal [22].

In addition to this Octanal, (E,E)-2,4-Heptadienal or Octanoic acid peaks are not present in the propylene glycol standard, neither in the support material used for solidification of the olive oil. This suggests that these compounds might have been extracted from the olive oil sample and been trapped in propylene glycol. The reason these compounds, including Nonanal appear in extraction attempt number three in higher amounts most probably has to do with the more severe extraction conditions of 45°C for 60 minutes and 200bar, as already

statet. Several papers suggest the detection of Nonanal as an appropriate method to detect initial oxidation [41, 42, 44, 45].

Frankel reported that Nonanal showed the highest rate of increment during oxidation of VOOs and considered it to be the most suitable index of the oxidation degree of olive oils [41, 49]. These results also stand in line with Morales et al. results who observed that more drastic conditions in SFE (higher temperature and pressure) achieved a volatile compound profile with more presence of off-flavours, by oxidising the olive oil samples [67].

3.2.3.1.2 20% Ethanol extracts

Figure 17 shows the GC-MS analysis results of the extracts using 20% ethanol as collecting solvent. Similar to the results obtained with propylene glycol, the extracts chromatogram are mostly equal to the 20% ethanol standard. Also here, the extract number four presents a higher peak in Nonanal because of the higher applied extraction temperature of 45°C and 100bar instead of the applied 37°C and 30°C and 80bar in other extracts.

Although the extracts number five and seven directly after extraction resembled an odour reminiscent of green olive or olive oil, before the GC-MS analysis this smell already had disappeared. Extract number nine shows a large peak of α-Farnesene at minute 28, this compound is present in the olive oil sample, as well as in the flour which was used as support material for the olive oil extraction, though in both cases the peaks are not even close to the extracts peak height. Since this large peak only appears in the extract attempt using flour as support matrix, it could be supposed that there is a relationship between the flour support matrix and this alpha-Farnesene peak.

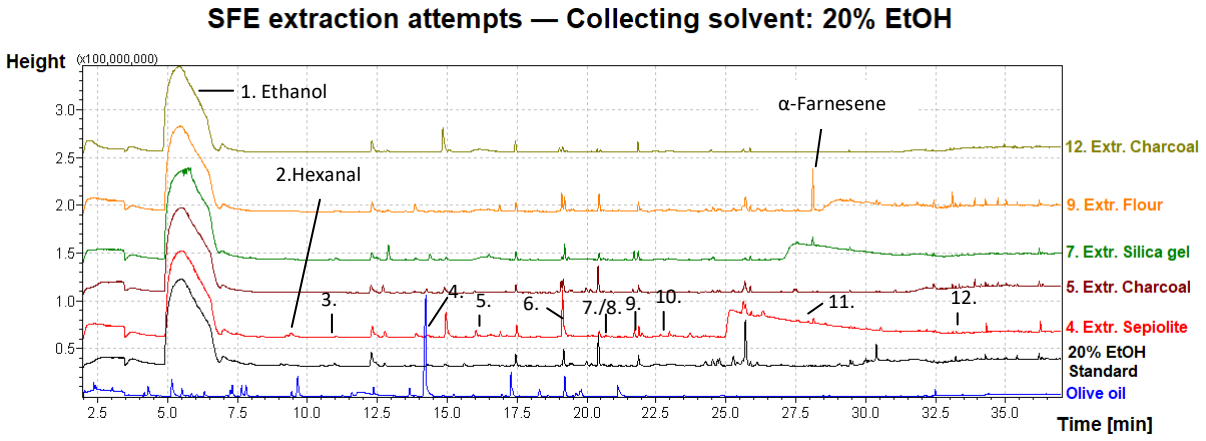
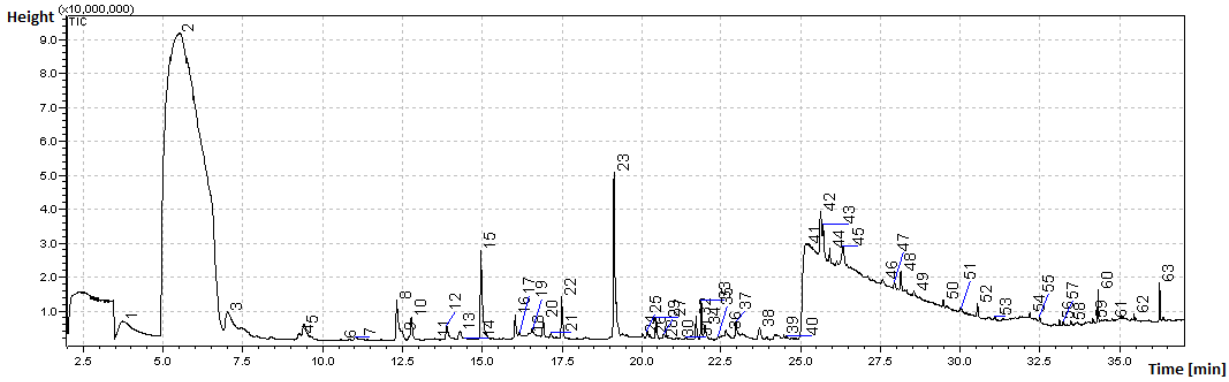


Figure 17: GC-MS analysis results of SFE extracts of olive oil sample, using 20% ethanol as collecting solvent. Peaks identified with sensory relevance: 1. Ethanol, 2. Hexanal, 3. Ethylbenzene, 4. (E)-2-Hexenal, 5. Octanal, 6. Nonanal, 7. Acetic acid, 8. E-E-2,4-Heptadienal, 9. α-Copaene, 10. Benzaldehyde, 11. α-Farnesene, 12. Octanoic acid.

All peaks appearing in extract number four are identified and listed below in Figure 18 compounds marked with a star, are of sensory relevance and also appear in the olive oil sample.



Number	Compound	RT	Area %	Similarity %
1	Cyclotrisiloxane, hexamethyl-	3.726	2.10	
2		5.508	82.37	
3	Cyclotetrasiloxane, octamethyl-	7.024	0.81	
4		9.256	0.06	
5 ☆	Hexanal	9.413	0.27	96
6 ☆	Ethylbenzene	10.571	0.01	90
7	Benzene, 1,3-dimethyl- (CAS) m-Xylene	11.021	0.05	
8	Cyclopentasiloxane, decamethyl-	12.317	0.55	
9	o-Xylene	12.457	0.02	
10	Tridecane	12.777	0.34	
11		13.501	0.02	
12 ☆	(E)-2-Hexenal	13.884	0.21	96
13	1-Dodecanol	14.308	0.17	
14	3-Ethyl-3-methylheptane	14.457	0.01	
15	Styrene	14.952	1.52	
16 ☆	Octanal	16.027	0.25	95
17	Tridecane	16.163	0.03	
18	1,5-Heptadiene, 3,3-dimethyl-, (E)- (CAS)	16.482	0.02	
19	2-Propanone, 1-hydroxy- (CAS) Acetol	16.557	0.06	
20	3-Hexen-1-ol, acetate, (Z)-	16.900	0.18	
21	Octane, 2,7-dimethyl- (CAS) 2,7-Dimethyloctane	17.146	0.06	
22	1,2-Bis(.gamma.-trimethylsilypropoxy)ethane	17.480	0.49	
23 ☆	Nonanal (CAS) n-Nonanal	19.125	2.20	95
24	1-Hexadecanol (CAS) Cetal	20.017	0.04	
25	2-Octenal, (E)-	20.158	0.07	
26	Octanoic acid, ethyl ester (CAS) Ethyl caprylate	20.274	0.02	
27	3-Hexadecene, (Z)-	20.476	0.23	
28	1-Dodecanol (CAS) n-Dodecanol	20.634	0.01	
29 ☆	Acetic acid	20.711	0.04	96
30 ☆	TRANS,TRANS-2,4-HEPTADIENAL	21.141	0.01	91
31	(+)-Cycloisosativene	21.409	0.03	
32 ☆	.alpha.-Copaene	21.729	0.24	93
33	Trisiloxane, 1,1,1,5,5,5-hexamethyl-3,3-bis[(trimethylsilyl)oxy]-	21.892	0.30	
34	2-Nonen-1-ol	22.008	0.14	
35	Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1R)-	22.411	0.02	
36 ☆	Benzaldehyde	22.659	0.09	96
37	2-Nonenal, (E)-	22.986	0.13	
38	1-Octanol (CAS) Octilin	23.719	0.22	
39	Caryophyllene	24.482	0.03	
40	Hexadecane	24.566	0.03	
41	1,2-Propanediol, 3-methoxy- (CAS) 3-Methoxy-1,2-propanediol	25.164	4.42	
42	Cyclohexanol, 1-methyl-4-(1-methylethyl)-	25.628	0.34	
43	2-Decenal, (Z)- (CAS) CIS-DEC-2-ENAL	25.707	0.11	
44	SILICATE ANION TETRAMER	25.913	0.10	
45	1-Hexacosanol	26.328	0.36	
46	.alpha.-Muurolene	27.572	0.03	
47		27.942	0.06	
48 ☆	.alpha.-Farnesene	28.140	0.16	96
49	2,4-Decadienal, (E,Z)- (CAS) trans,cis-2,4-Decadienal	28.545	0.05	
50	SILICATE ANION TETRAMER	29.474	0.05	
51	2-Propanol, 1,1'-oxybis-	30.024	0.05	
52	5,9-Undecadien-2-one, 6,10-dimethyl-, (Z)-	30.543	0.13	
53	Butyric acid, 4-tridecyl ester	31.085	0.04	
54	SILICATE ANION TETRAMER	32.183	0.03	
55	1-Dodecanol (CAS) n-Dodecanol	32.491	0.04	
56	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, (E)-	33.111	0.03	
57 ☆	Octanoic Acid	33.227	0.03	92
58	SILICATE ANION TETRAMER	33.468	0.02	
59	Nonanoic acid	34.158	0.03	
60	Phenol, 5-methyl-2-(1-methylethyl)- (CAS) Thymol	34.316	0.15	
61	1H-Indene, 2,3-dihydro-1,1,3-trimethyl-3-phenyl-	34.765	0.01	
62	Phenol, 2,4-bis(1,1-dimethylethyl)-	35.450	0.04	
63	Diethyl Phthalate	36.246	0.27	

Figure 18: GC-MS analysis results of SFE extraction attempt number four, using 20% ethanol as collecting solvent (marked with star, compounds of sensory relevance also appearing in olive oil sample).

Comparing the extraction results of Table 15 using propylene glycol and Table 16 using 20% ethanol as collecting solvent it becomes clear that even when applying the same extraction conditions as done in extraction attempt number five and six, peak areas and number of compounds trapped are distinguishably higher in the attempt using 20% ethanol as collecting solvent than using propylene glycol.

Similar to the results already observed in extraction attempt number three using propylene glycol as collecting solvent, applying a higher pressure for extraction of 100bar in comparison to 80bar in attempt number four resulted in higher peak areas for trapped compounds, especially for Nonanal.

Table 16: Overview of extraction results and extraction conditions using 20% ethanol as collecting solvent, peak areas in percent (marked in red, peak areas standing out).

	Peak areas %									
Compounds	**Olive oil**	**Silica gel**	**Sepiolite**	**Charcoal**	**Flour**	**4. Extr. Sepiolite**	**5. Extr. Charcoal**	**7. Extr. Silica gel**	**9. Extr. Flour**	**12. Extr. Charcoal**
Hexanal	2,75	-	-	-	2,98	0,27	-	-	-	-
(E)-2-Hexenal	38,66	-	-	-	-	0,21	0,05	0,12	0,48	0,05
Ethylbenzene	0,01	-	0,71	-	-	0,01	-	0,01	-	0,06
Octanal	0,02	-	-	-	-	0,25	0,06	-	0,04	0,02
Nonanal	0,13	0,04	0,09	-	1,93	2,20	0,30	0,09	0,61	0,15
Acetic acid	4,73	0,15	0,18	-	1,47	0,04	0,04	0,03	-	0,10
(E,E)-2,4-Heptadienal	0,03	-	-	-	-	0,01	0,00	-	0,00	-
alpha-Copaene	0,18	-	-	-	-	0,24	0,14	-	0,03	0,01
Benzaldehyde	0,03	-	-	-	-	0,09	0,01	0,02	-	0,03
alpha-Farnesene	0,18	-	-	-	0,10	0,16	0,03	0,31	1,66	0,02
Octanoic Acid	0,04	-	-	-	-	0,03	-	-	-	-
Extraction conditions						45°C, 100 bar, 30min dynamic, 2min static, Sepiolite, 35ml 20% EtOH	37°C, 80 bar, 30min dynamic, 2min static, Charcoal, 35ml 20% EtOH	37°C, 80 bar, 30min dynamic, 2min static, Silica gel, 25ml 20% EtOH	37°C, 80 bar, 30min dynamic, 3:30min static, Sepiolite, 25ml 20% EtOH	30°C, 80 bar, 30min dynamic, 2min static, Charcoal, 40ml 20% EtOH

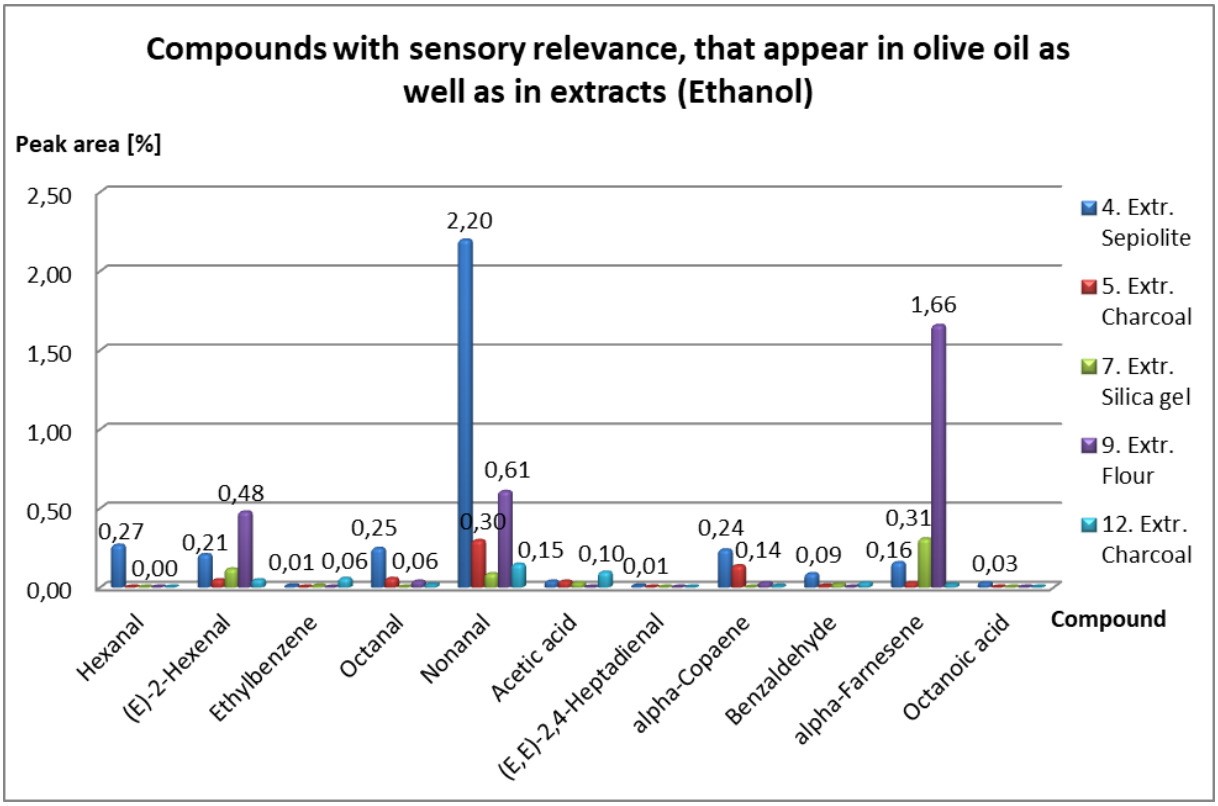


Figure 19: Graphical comparison of extraction results of SFE extraction, using 20% ethanol as collecting solvent.

3.2.3.1.3 Refined oil extracts

Figure 20 shows the GC-MS analysis results of the extracts using refined oil as collection liquid. Noticeable here is the larger peak of acetic acid in both extracts. This compound is both present in the refined oil, as well as in the olive oil sample, but in both cases does not even have a comparable large peak. The peak in extract number eleven has the highest peak, what suggests that the higher temperature of 37°C in comparison to 30°C might be responsible for a higher amount of Acetic acid in the extract, as for GC-MS analysis there was analysed the same quantity of each extract.

Although milder extraction conditions were used in these attempts, extraction results in terms of number of compounds identified and peak areas are higher using refined oil as collection liquid, compared to propylene glycol, but still lower than using 20% ethanol.

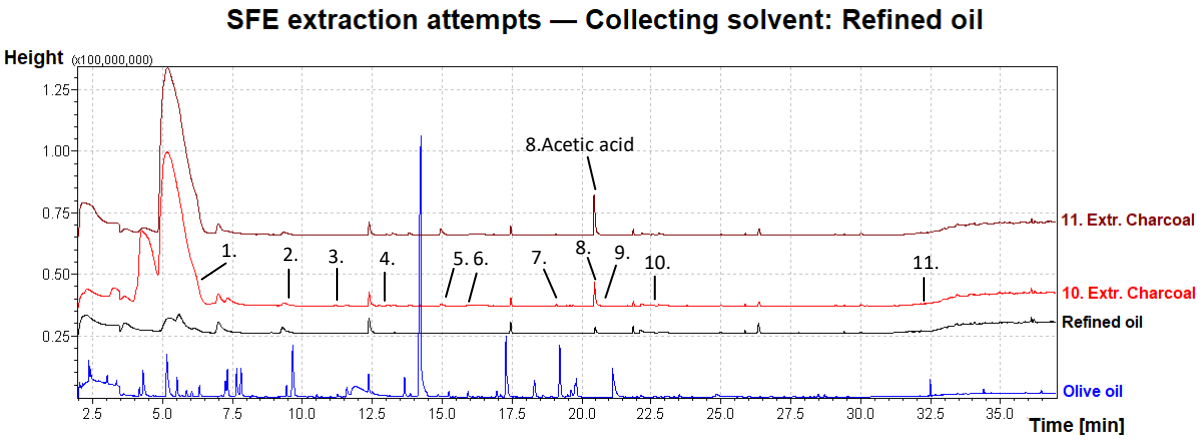
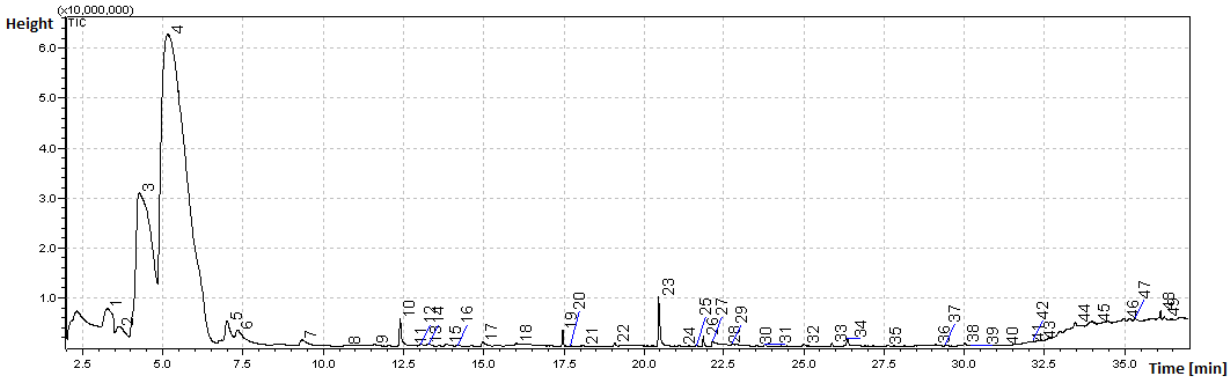


Figure 20: GC-MS analysis results of SFE extracts of olive oil sample, using refined oil as collecting solvent. Peaks identified with sensory relevance: 1. Ethanol, 2. Hexanal, 3. Ethylbenzene, 4. Heptanal, 5. (E)-2-Hexenal, 6. Octanal, 7. Nonanal, 8. Acetic acid, 9. 2-Butanaone, 10. Benzaldehyde, 11. Heptanoic acid.

All peaks appearing in extract number ten are identified and listed below in Figure 21 compounds marked with a star, are of sensory relevance and also appear in the olive oil sample.



Number	Compound	RT	Area %	Similarity %
1	2-Propanone (CAS) Acetone	3.269	1.93	
2	Cyclotrisiloxane, hexamethyl-	3.629	0.54	
3	Methanol (CAS) Carbinol	4.259	19.09	
4 ☆	Ethanol	5.172	73.11	96
5	Cyclotetrasiloxane, octamethyl-	7.002	0.87	
6	Chloroform	7.328	0.50	
7 ☆	Hexanal	9.327	0.33	94
8 ☆	Ethylbenzene	10.699	0.03	91
9		11.577	0.05	
10	Cyclopentasiloxane, decamethyl-	12.392	0.67	
11 ☆	Heptanal (CAS) n-Heptanal	12.747	0.02	92
12	l-LIMONENE	13.035	0.06	
13	Undecane, 2,6-dimethyl- (CAS) 2,6-Dimethylundecane	13.231	0.01	
14	Eucalyptol	13.301	0.02	
15 ☆	2-Hexenal, (E)-	13.825	0.08	91
16	Furan, 2-pentyl-	14.195	0.02	
17	Styrene	14.959	0.18	
18 ☆	Octanal (CAS) n-Octanal	16.017	0.01	88
19	1,2-Bis(.gamma.-trimethylsilypropoxy)ethane	17.454	0.22	
20		17.707	0.01	
21	2-Pentanone, 4-hydroxy-4-methyl-	18.091	0.01	
22 ☆	Nonanal (CAS) n-Nonanal	19.069	0.07	94
23 ☆	Acetic acid (CAS) Ethylic acid	20.478	0.99	98
24 ☆	2-Butanone	21.112	0.01	87
25	3-Cyclopentene-1-acetaldehyde, 2,2,3-trimethyl-	21.655	0.02	
26	Trisiloxane, 1,1,1,5,5,5-hexamethyl-3,3-bis[(trimethylsilyl)oxy]-	21.875	0.13	
27	Formic acid (CAS) Bilorin	22.161	0.20	
28 ☆	Benzaldehyde	22.558	0.02	90
29	Propanoic acid (CAS) Propionic acid	22.780	0.03	
30	Propanedioic acid, dimethyl-	23.539	0.01	
31	Propanoic acid, 2,2-dimethyl-	23.782	0.03	
32		25.008	0.05	
33	SILICATE ANION TETRAMER	25.873	0.04	
34		26.361	0.16	
35 ☆	Pentanoic acid	27.594	0.01	
36	Ethanol, 2-(2-butoxyethoxy)- (CAS) 2-(2-Butoxyethoxy)ethanol	29.102	0.02	
37	SILICATE ANION TETRAMER	29.420	0.02	
38	Hexanoic acid (CAS) n-Hexanoic acid	30.005	0.06	
39	Ethanol, 2-(2-butoxyethoxy)-, acetate	30.217	0.01	
40		31.208	0.01	
41 ☆	Heptanoic acid	32.011	0.02	90
42		32.149	0.02	
43	Ethanol, 2,2'-oxybis- (CAS) Diethylene glycol	32.390	0.02	
44		33.444	0.09	
45	Nonanoic acid	34.062	0.02	
46	Ethanol, 2,2'-[oxybis(2,1-ethanediyloxy)]bis-	34.972	0.03	
47		35.332	0.02	
48	Diethyl Phthalate	36.107	0.08	
49	1-Hexadecanol (CAS) Cetal	36.226	0.05	

Figure 21: GC-MS analysis results of SFE extraction attempt number ten, using refined oil as collecting solvent (marked with star, compounds of sensory relevance also appearing in olive oil sample).

Table 17 gives a general overview of the extraction results of sensory relevant compounds and extraction conditions used, using refined oil as collection liquid.

The subsequent following Figure 22 shows a graphical comparison of the extraction results illustrated in Table 17.

Noticeable is that the compounds Hexanal and Acetic acid have the highest peak areas in the extracts. It is most likely that this in the biggest part due to the fact that the used collection liquid, refined oil, itself has high peak areas of these compounds, rather than a consequence of extraction and trapping from the olive oil sample. As can be taken from the following Table 17. As already mentioned in extract number eleven, the peak area of Acetic acid might be higher because of the higher applied extraction temperature on the olive oil sample.

Table 17: Overview of extraction results and extraction conditions using refined oil as collecting solvent, peak areas in percent (marked in red, peak areas standing out).

	Peak areas %				
Compounds	**Olive oil**	**Refined oil**	**Charcoal**	**10. Extr. Charcoal**	**11. Extr. Charcoal**
Hexanal	2,75	6,65	-	0,33	0,32
Ethylbenzene	0,01	-	-	0,03	-
Heptanal	0,02	0,16	-	0,02	0,01
(E)-2-Hexenal	38,66	-	-	0,08	0,15
Octanal	0,02	0,11	-	0,01	-
Nonanal	0,13	0,23	-	0,07	0,05
Acetic acid	4,73	2,58	-	0,99	1,55
2-Butanone	0,03	-	-	0,01	0,00
Benzaldehyde	0,03	0,06	-	0,02	0,02
Pentanoic acid	0,01	0,07	-	0,01	0,02
Heptanoic acid	0,06	-	-	0,02	-
Extraction conditions				30°C, 80 bar, 30min dynamic, 2min static, charcoal, 20ml refined oil	37°C, 80 bar, 30min dynamic, 2min static, charcoal, 25ml refined oil

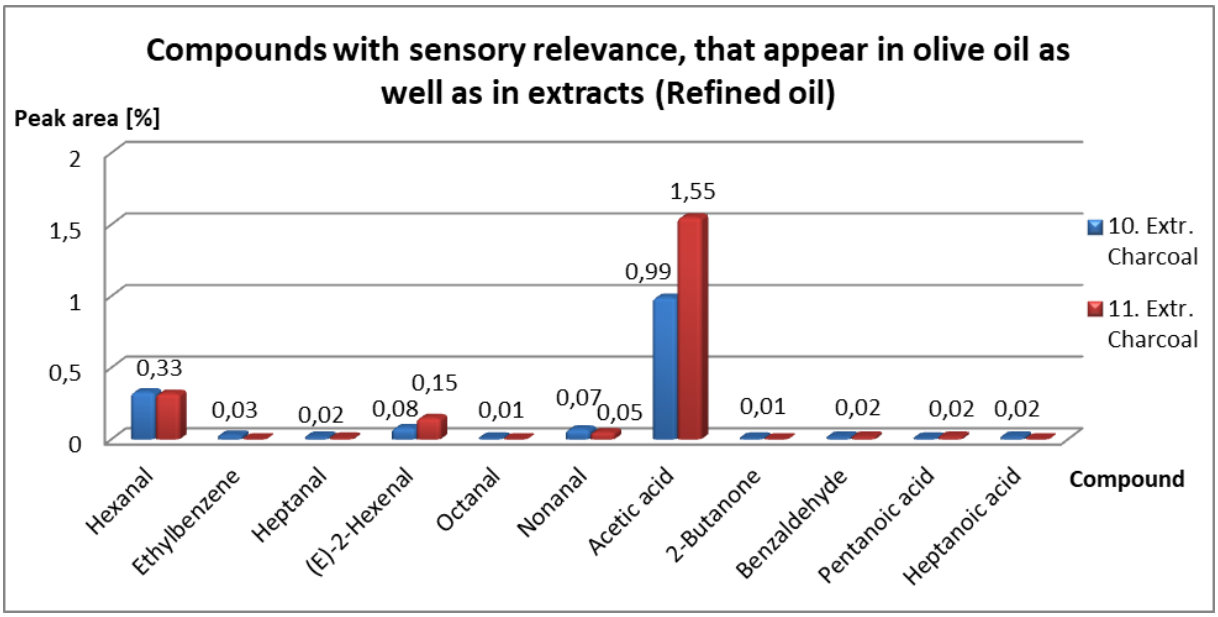


Figure 22: Graphical comparison of extraction results of SFE extraction, using refined oil as collecting solvent.

3.2.4 Conclusion SFE

Using supercritical fluid extraction, directly after extraction there was no odour resembling olive or olive oil odour observed in the extracts, except for extract number five and seven, which had faded away before GC-MS analysis. Although the olfactory evaluation cannot be at all an indication for no collection of volatile compounds, neither for compounds extracted from the olive oil sample, nor from compounds extracted from the support matrix used for extraction, into the extracts. As the extracted compounds concentration can be too low to be recognized by sensorial evaluation.

The GC-MS analysis results show that it was in fact possible to extract and trap some volatile compounds of the olive oil sample in the tested collecting solvents. Although results seem to be poor and mostly in trace quantities.

Comparing the three tested collection liquids propylene glycol, 20% ethanol and refined oil, 20% ethanol in number of compounds trapped and peak areas, showed to be the most effective collection liquid for trapping of the olive oil volatile compounds.

Interesting is the fact that in all extracts mostly compounds of sensory relevance from the olive oil could be trapped, that are in relationship with oxidation of the olive oil [1. 18, 49] .

Namely compounds like Octanal, Nonanal, (E,E)-2,4-Heptadienal, Octanoic acid, Heptanal and Heptanoic acid. In spite of the fact that these compounds are only present in trace amount in the extracted olive oil sample of good quality.

Exceptions are the compounds (E)-2-Hexenal which is by far the most abundant volatile compound present in the extracted olive oil sample and is considered as a positive contributor to the mostly as green, fruity, cut-grass and as bitter almond described odours [54, 55, 81, 82]. And Acetic acid, present in traces in the extracts, which is responsible for undesirable sour, vinegary and pungent, acetic acid like odours [53, 83, 84].

In general terms, more drastic extraction conditions (higher temperature and pressure) lead to higher peak areas and more compounds extracted to the extracts.

Although charcoal and 10% ethanol as co-solvent were applied to overcome the suspicion of a low extraction capacity of the polar volatile compounds, because of the CO_2s low polarity, in GC-MS results, no correlation or relationship between selective extraction of compounds from the olive oil sample by means of the used support material or co-solvent for olive oil extraction was possible to be observed. However, after applying these measures directly after extraction it was possible to recognize a chemical, slightly green olive reminiscent odour in two extracts, using 20% ethanol as collection liquid, which was disturbed by the ethanol smell and had faded away again before GC-MS analysis.

Regarding these results, it should not be forgotten the fact that the quantitative extraction conditions cannot be developed and evaluated unless the collection step is efficient [59].

Considering this fact and the poor extract results, at this point it is possible to build two hypotheses:

1. The extraction step was not as successful as intended;
2. The conditions used for the trapping of the compounds were not the most appropriate or effective enough for trapping the target analytes. In fact, the success of an extraction method depends not only on the extraction step itself, but also on the matrix considered and the analyte trapping system [59].

Comparing the SFE results obtained with the ones from Morales et al. work, who used a solid-phase Tenax TA trap for trapping of the volatile compounds from an virgin olive oil sample with thermal desorption for GC-MS analysis, in the present work a smaller number of volatile compounds were able to be extracted or trapped. Whilst Morales et al. were able to identify 21 compounds of sensory relevance with GC-MS with higher percentual peak areas, the maximum in this work was SFE extract number four with eleven compounds, mostly only in trace values with a more off-flavour compounds weighted profile [67].

3.3 Distillation

Distillation is one of the most commonly used techniques for the isolation of volatile substances of foods. The methods most commonly used are vacuum and steam distillation (SD). Reduced pressure distillation is of great interest, as the working temperature is lower, thus minimizing possible changes in the sample. Generally, the vapours from distillation are condensed on a refrigerant or trapped in different cryogenic traps or adsorbent materials (although the latter is less frequent) [1].

In the production of essential oils and generally of volatile compounds, steam distillation often proves to be very useful, although it involves an exposure to heat that may cause degradation of thermally labile molecules [85].

For gentle evaporation of volatiles, the rotary evaporator is used in the laboratory. The rotating distillation flask creates high turbulence and a new thin film in the upper part of the flask with every rotation. This allows a high heat and mass transfer rate and overheating of the liquid is prevented [86].

Hydrodistillation (SD) has been applied for the analysis of leaf, fruit and virgin oil volatiles of an Italian olive cultivar by Flamini et al. by means of a Clevenger-type apparatus (see Figure 23) [30, 88]. Thereby, the oil and water phases are mixed in a flask and subsequently distilled; this operation can also be carried out in a rotary evaporator [89].

The uptake of olive oil volatiles in Flamini et al. experiment was comparable to that obtained by SPME using a PDMS fiber. However, with hydrodistillation the volatiles in the steam distillate are heavily diluted by water when collected in cold traps. In comparison with the extraction of the same oil by SPME and CLSA, SDE gave higher percentages of aldehydes correlated with the oxidative degradation of VOO, indicating that the extraction conditions induced the thermal alteration of the oil sample. The water dilution in hydrodistillation is overcome in Simultaneous Distillation-Extraction (SDE) via solvent extraction of the distillate [30, 88].

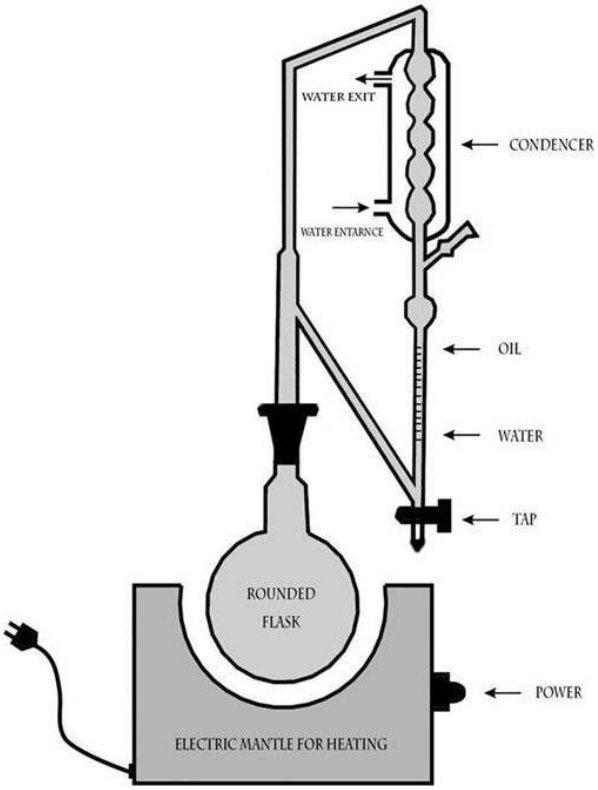


Figure 23: Schematic diagram of Clevenger apparatus set up used for hydrodistillation [90].

Weurman used Simultaneous Distillation-Extraction (SDE) (see Figure 24) to obtain volatile compounds from fats and oils. This method, introduced by Likens and Nickerson in 1964, consists of separate distillations of a dilute aqueous solution of the sample and the solvent (ether, pentane, dichloromethane). They condense in the same area where extraction takes place. The two phases are then separated and recycled. It also has disadvantages, such as not being appropriate for thermolabile volatile compounds and the use of toxic solvents. Its use with oil samples is infrequent [1, 89].

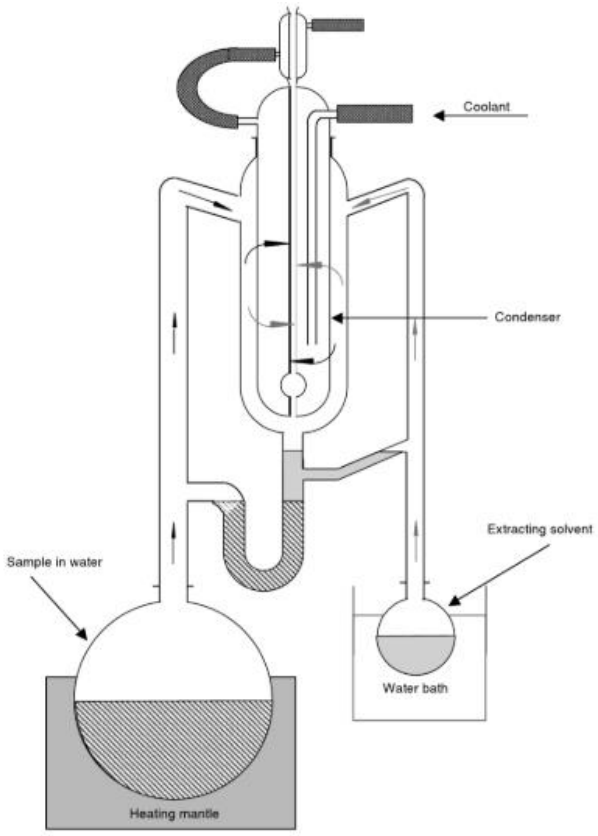


Figure 24: Likens-Nickerson apparatus for simultaneous distillation-extraction [91].

Flath and co-workers carried out an exhaustive study of the volatile compounds of virgin olive oil. They used codistillation with water, followed by solvent extraction and dry-column chromatography, to obtain a polar concentrate and identified seventy-seven volatile compounds by using GC-MS analysis. The organoleptic assessment of some of these compounds indicated that several were significant contributors to aroma [1].

Among distillation and fluid-based extraction techniques, only hydrodistillation, SDE and SFE have been tentatively applied for the analysis of VOO aroma [30].

3.3.1 Equipment and materials

The following equipment and materials were used for the olive oil extraction using the distillation process:

Table 18: Atmospheric and vacuum distillation: equipment and materials.

Atmospheric distillation	LBX Instruments Digital magnetic stirrer with heating and ceramic coated plate, LBX H03D. Distillation apparatus as depicted in Figure 25 with silicone bath.
Vacuum distillation	BÜCHI Rotavapor R-114; BÜCHI Waterbath B-480; BÜCHI Vacuum Pump V-700 with Vacuum Controller V-850
Materials	Small bucket with ice, 20% ethanol, Propylene glycol: Fagron – Propylenglycolum (76,1) $C_3H_8O_2$ d. = 1,038

3.3.2 Sample preparation and distillation conditions

Hydrodistillation was performed as it is a non-toxic solvent extraction method, in atmospheric conditions on a distilling apparatus, as well as in vacuum conditions on a rotary evaporator. Distillation conditions with hydrodistillation performed by Flamini et al. on Clevenger-type apparatus (Figure 23) were not very detailed.

Research done on boiling point temperatures of most common volatile compounds showed high boiling point temperatures of plus 100°C. Among the contributors to high quality olive oil, the most important ones, besides (Z)-3-Hexenal (106-122°C), are (Z)-3-Hexen-1-ol (156°C) and Hexanal (103-131°C) and (E)-2-Hexenal (91-171°C) [92].

For atmospheric distillation, the applied distillation conditions together with observations are shown in Table19. In a first trial 20ml olive oil were mixed for this purpose in the extraction flask together with 25ml 20% ethanol. Additionally 10ml 20% ethanol were put in collection flask to serve as collection solvent. The extraction flask was heated in a silicone bath. Precautions must be taken to ensure efficient condensation of the steam and collection of the condensate, in such a way as to prevent loss of the volatile material. However, to avoid risk of explosion, a completely closed system must not be used [87, 95].

For this purpose, the end of the distillation apparatus was immersed directly into the collection solvent in the collection flask, similar as to already illustrated before in Figure 10. The collection flask was kept in ice during distillation to help prevent volatilization of volatile compounds. Experimental setup is shown in Figure 25.

Figure 25: Experimental setup for hydrodistillation of olive oil sample.

Vacuum distillation was performed on a common rotary evaporator (see Table 18). Used distillation conditions together with observations are shown in Table 19 and 20. Besides using 20% ethanol as solvent, some trials using propylene glycol were performed.

All extracts after the distillation processes were stored in the fridge and subsequently analysed by GC-MS under the same analysis conditions as used for the olive oil samples in Chapter 2.2.

Table 19: Atmospheric distillation of olive oil, experimental conditions and observations.

Atmospheric distillation		
	1. Extr.	**2. Extr.**
Olive oil	20 ml	20 ml
Solvent	25 ml 20% EtOH mixed with olive oil, 10 ml 20% EtOH in collection flask	25 ml 20% EtOH mixed with olive oil, 5 ml 20% EtOH in collection flask
Temp. heating plate	100°C first 15min, then 130°C	150°C
Time	1h 25min	1h 30min
Observation	Seemed to have not much smell	Not much smell, a strange green odour

Table 20: Vacuum distillation of olive oil on rotary evaporator, experimental conditions and observations.

Vacuum distillation				
	1. Extr.	**2. Extr.**	**3. Extr.**	**4. Extr.**
Olive oil	25ml	20 ml	20 ml	20 ml
Solvent	First hour without solvent, rest of time 30 ml 20% ethanol mixed in olive oil	15 ml PG in collection flask	10 ml water mixed in olive oil and 10 ml PG in collection flask	20 ml 20% EtOH mixed with olive oil, 10 ml PG in collection flask
Temp. water bath	40°C	40°C	40°C	40°C
Pressure	15-20 bar	15-20 bar	15-20 bar	15-20 bar
Extraction time	2h	30 min	1h	1h
Observation	Little smell	Nearly no smell, only the flask had smell	Very little smell like olive oil of the extract	Very little to no smell

3.3.3 Results and discussion

Different distillation conditions in atmospheric, as well as in vacuum conditions were tested and extraction conditions and extracts observations are shown in Table 19 and Table 20. None of the extracts resemble though the original scent of the fresh green olive oil sample. Rather it was noticeable in some extracts, that the high temperature had some detrimental effect on the smell sensation, although there was no oxidised or rancid smell.

In the following chapter are discussed the GC-MS analysis results of the obtained extracts.

3.3.3.1 GC-MS analysis results of extracts

3.3.3.1.1 Atmospheric distillation

Looking on the chromatographic results of the extracts of the distillation under atmospheric conditions in Figure 26 it is clear to see, that besides of Nonanal (number seven), it was possible to extract and trap a large peak of (E)-2-Hexenal (number five). (E)-2-Hexenal is the most abundant volatile compound in European, Tunisian and Moroccan olive oils and is also the most abundant volatile compound in the used olive oil sample [49].

C6 aldehydes like Hexanal and (E)-2-Hexenal, as well as Hexanol, contribute to the typical green sensory perception.

As already mentioned before in Chapter 2.4, the Hexanal/(E)-2-Hexenal ratio is a very important indicator of the freshness of the oils and can be used to estimate their oxidation de-

gree. High quality oils show higher (E)-2-Hexenal levels than Hexanal. When the oil oxidation is induced, a fast increase of Hexanal and a decrease of (E)-2-Hexenal levels takes place, and then a “rancid” off-flavour appears [46]. (E)-2-Hexenal boiling point lays according to FooDB at 91°C and according to Alfa Aesar at 171°C [92].

Although the (E)-2-Hexenal peaks of the extracts are slightly offset in their retention time, from the (E)-2-Hexenal peak of the olive oil sample, peaks of extract number one presents a similarity of 93% with GC-MS library and the peak of extract number two a similarity of 95%. Practical kovats showed to be identical with the theoretical ones found in literature.

Extract number one shows a much larger peak in (E)-2-Hexenal and Nonanal than extract number two, whilst extract number two has a large peak of alpha-Copaene. Both of the extracts present a large peak of alpha-Farnesene. Alpha-Farnesene odour is described amongst others as floral, green plant and could have been together with (E)-2-Hexenal or alpha-Copaene responsible for the strange green smell observed after distillation [79].

Cecchi and Alfei found that Copaene, whose flavour is described as woody is positively correlated with the sum of artichoke and tomato notes, while alpha-Farnesene is characterised by herbal and green notes [17].

Flamini, Cioni and Morelli applied hydrodistillation for 2 hours on virgin olive oil and reported that (E,E)-α-Farnesene was the main constituent in hydrodistillation analysis with 25.0%, whilst with SPME analysis it was present only at 1.0% levels. The authors pointed out that SPME technique should better represent the real profile of volatiles emitted spontaneously at room temperature by the virgin olive oil [88].

Works from Vichi et al. and from Caja et al. applying simultaneous distillation-extraction (SDE) for the analysis of VOO aroma, detected as well a higher percentage in alpha-Farnesene [39, 93].

Morales, Rios and Aparicio studied the change of the volatile composition of virgin olive oil along the oxidation process by using an accelerated process. The authors observed that once the initial volatiles had disappeared, the concentration of certain volatiles increased. In their study 2-Farnesene greatly increased after 5 hours of oxidation. During the subsequent hours, the concentrations of several aldehydes increased, amongst others volatiles that were shown to be present in the obtained extracts such as Hexanal, Nonanal and Octanal. Almost all these volatiles are responsible for VOO off-flavours because their threshold levels for odour are very low [1].

Similar to this work, Nunes et al. evaluated the effect of heating on the volatile composition of extra-virgin olive oil. The results confirmed that aldehydes, like the ones before mentioned in addition to Hexanoic acid and Octanoic acid were more abundant in olive oil heated at 150°C [49]. Rancid oils are characterized by volatiles coming from the oxidation of unsaturated fatty acids, mainly aldehydes like Pentanal, Hexanal, Heptanal, Octanal, Nonanal and acids in e.g., Acetic, Butanoic, Hexanoic, and Heptanoic acid [18].

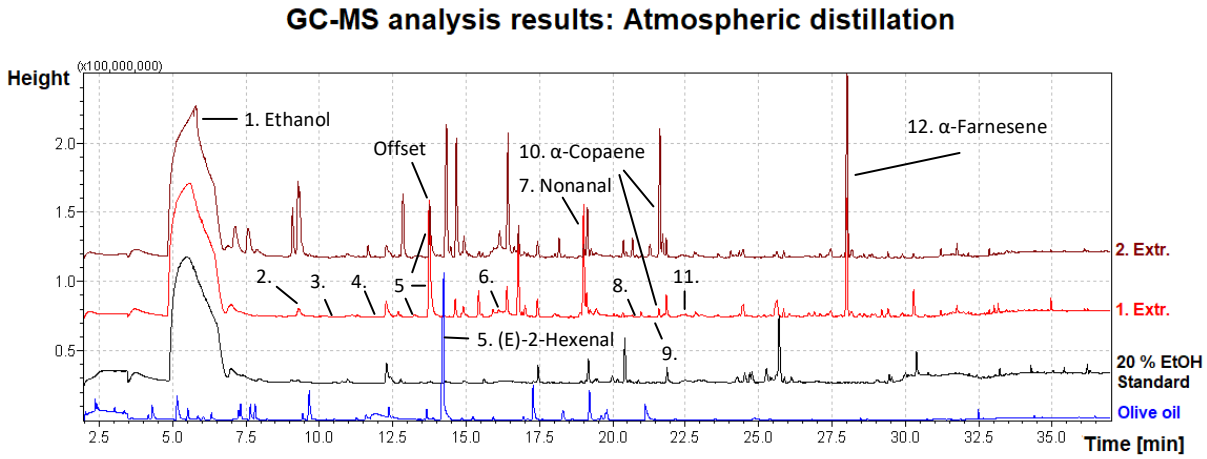
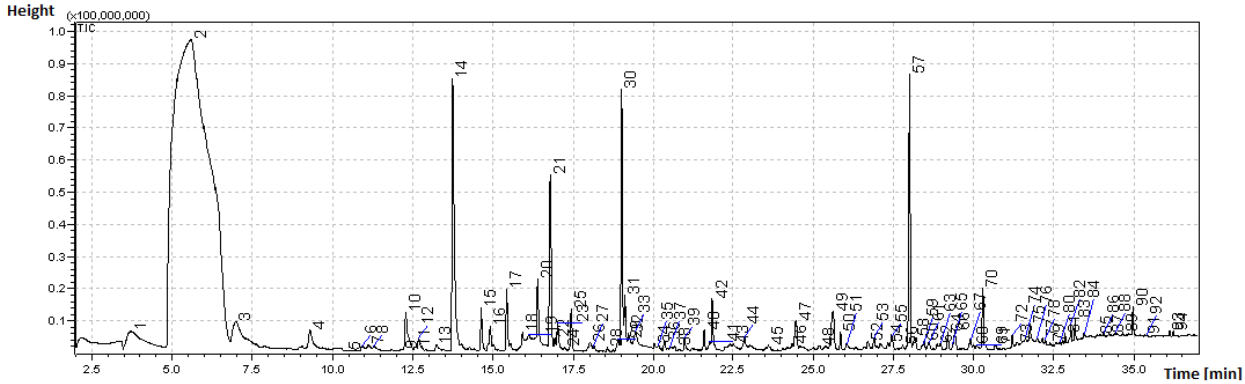


Figure 26: GC-MS analysis results of the extracts of atmospheric distillation of the olive oil sample. Peaks identified with sensory relevance: 1. Ethanol, 2. Hexanal, 3. Ethylbenzene, 4. 1-Penten-3-ol, 5. (E)-2-Hexenal, 6. Octanal, 7. Nonanal, 8. Acetic acid, 9. (E,E)-2,4-Heptadienal, 10. Benzaldehyde, 11. α-Farnesene.

All peaks appearing in extract number one are identified and listed below in Figure 27. Compounds marked with a star, are of sensory relevance and also appear in the olive oil sample.



Number	Compound	RT	Area %	Similarity %
1	Cyclotrisiloxane, hexamethyl-	3.716	1.94	
2		5.597	74.83	
3	Cyclotetrasiloxane, octamethyl-	6.978	0.83	
4 ☆	Hexanal	9.298	0.41	93
5 ☆	Benzene, ethyl- (CAS) EB	10.420	0.00	93
6	Benzene, 1,3-dimethyl- (CAS) m-Xylene	10.924	0.07	
7 ☆	4-Pentenal, 2-methyl-	11.110	0.07	92
8 ☆	3-Hexenal, (Z)-	11.287	0.06	94
9 ☆	1-Penten-3-ol	12.159	0.01	88
10	Cyclopentasiloxane, decamethyl-	12.277	0.73	
11	Cyclopentasiloxane, decamethyl-	12.563	0.02	
12	l-LIMONENE	12.684	0.10	
13 ☆	2-Hexenal, (E)-	13.235	0.11	92
14 ☆	2-Hexenal, (E)-	13.741	4.26	93
15	1,3,6-Octatriene, 3,7-dimethyl-, (Z)-	14.628	0.49	
16	Cyclohexane, (1-methylethylidene)-	14.907	0.27	
17	Acetic acid, hexyl ester	15.424	0.58	
18 ☆	Octanal	15.916	0.13	95
19	Pentadecane	16.105	0.07	
20	1,5-Heptadiene, 3,3-dimethyl-, (E)-	16.389	0.56	
21	3-Hexen-1-ol, acetate, (E)- (CAS) TRANS-HEX-3-ENYL ACETATE	16.778	2.08	
22	3-Hexen-1-ol, acetate, (Z)-	16.916	0.05	
23	2-Heptenal, (Z)- (CAS) CIS-HEPT-2-ENAL	17.005	0.24	
24	Benzene, 1-ethyl-3-methyl-	17.227	0.03	
25	Malonic acid, bis(2-trimethylsilylethyl ester	17.428	0.43	
26 ☆	1-Hexanol	18.018	0.10	96
27	Decane, 3,8-dimethyl-	18.143	0.03	
28	Benzene, 1,3,5-trimethyl-	18.551	0.03	
29		18.898	0.10	
30 ☆	Nonanal	19.005	2.26	97
31	Dodecane	19.105	0.30	
32	Cyclopropane, 1,1-dimethyl-2-(2-methyl-2-propenyl)-	19.219	0.09	
33 ☆	2-Hexen-1-ol, (E)-	19.434	0.23	93
34	2-Octenal, (E)- (CAS) trans-2-Octenal	20.024	0.03	
35	Octanoic acid, ethyl ester	20.149	0.02	
36	1-Tetradecene (CAS) n-Tetradec-1-ene	20.372	0.10	
37 ☆	Acetic acid (CAS) Ethylic acid	20.539	0.01	97
38	.alpha.-Cubebene	20.690	0.03	
39 ☆	TRANS,TRANS-2,4-HEPTADIENAL	20.993	0.13	96
40 ☆	.apha.-Copaene	21.607	0.17	95
41 ☆	2,4-Heptadienal, (E,E)-	21.783	0.02	91
42		21.860	0.38	
43 ☆	Benzaldehyde	22.515	0.02	95
44	Heptadecanoic acid, ethyl ester (CAS) Ethyl n-heptadecanoate	22.856	0.08	
45	Decane, 3,8-dimethyl- (CAS) 3,8-Dimethyldecane	23.609	0.02	
46	Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-	24.344	0.03	
47	Hexadecane	24.482	0.43	
48	7-Hexadecene, (Z)-	25.189	0.07	
49	9-Octadecene, (E)- (CAS)	25.628	0.80	
50	SILICATE ANION TETRAMER	25.855	0.14	
51	2-OCTENE, 3,7-DIMETHYL-, CIS/TRANS	26.052	0.04	
52	1,4-Methanoazulene-9-methanol, decahydro-4,8,8-trimethyl-, [1S-(1.alpha.,3a.beta.,4.alpha.,8a.beta.,9R@)]-	26.707	0.07	
53	Hexadecane	26.899	0.09	
54	5-Octadecene, (E)-	27.334	0.04	
55	trans-.alpha.-Bergamotene	27.456	0.16	
56		27.793	0.03	
57 ☆	.alpha.-Farnesene	28.004	2.94	96
58	.DELTA.-CADINENE	28.163	0.17	
59		28.425	0.01	
60	Heptadecane, 3-methyl- (CAS) 3-Methylheptadecane	28.453	0.03	
61		28.607	0.09	
62	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-, (1.alpha.,4a.alpha.,8a.alpha.)- (CAS) .gamma.	28.866	0.04	
63	8-Hexadecenal, 14-methyl-, (Z)-	28.973	0.04	
64	Hexadecane	29.199	0.17	
65	Cyclopentane, 1-pentyl-2-propyl-	29.360	0.02	
66	SILICATE ANION TETRAMER	29.410	0.16	
67	5-Octadecene, (E)-	29.905	0.12	
68	4-Undecene, 3-methyl-, (Z)-	29.998	0.03	
69	Hexadecane, 1,16-dichloro-	30.096	0.02	
70	9-Eicosene, (E)-	30.286	0.63	
71	1-Decene, 3,3,4-trimethyl-	30.677	0.05	
72	Tricyclo[3.1.0.0(2,4)]hexane, 3,6-diethyl-3,6-dimethyl-, trans-	31.207	0.09	
73	Eicosane	31.359	0.03	
74		31.617	0.03	
75	Tricyclo[3.1.0.0(2,4)]hexane, 3,6-diethyl-3,6-dimethyl-, (E)-	31.760	0.12	
76	DEHYDROAROMADENDRENE	31.978	0.03	
77	SILICATE ANION TETRAMER	32.144	0.02	
78	t-Butyl isobutyl ketone	32.236	0.03	
79	1-Dodecanol (CAS) n-Dodecanol	32.380	0.01	
80	Eicosane (CAS) n-Eicosane	32.688	0.02	
81		32.856	0.05	
82	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, (E)-	33.027	0.08	
83	1-Nonadecene (CAS)	33.164	0.16	
84		33.440	0.04	
85	Octadecane, 1-(ethenyloxy)-	33.844	0.01	
86	Hexadecanoic acid, ethyl ester (CAS) Ethyl palmitate	34.055	0.03	
87	THYMOL	34.213	0.01	
88	Ethanol, 2-[2-(2-butoxyethoxy)ethoxy]-	34.440	0.01	
89	1H-Indene, 2,3-dihydro-1,1,3-trimethyl-3-phenyl- (CAS) 1,1,3-Trimethyl-3-phenylindan	34.651	0.02	
90	Hexadecanoic acid, ethyl ester	34.962	0.18	
91	Phenol, 2,4-bis(1,1-dimethylethyl)- (CAS) 2,4-Di-tert-butylphenol	35.332	0.01	
92		35.400	0.01	
93	Diethyl Phthalate	36.105	0.03	
94	1-Hexadecanol (CAS) Cetal	36.215	0.04	

Figure 27: GC-MS analysis results of atmospheric distillation attempt number one, using 20% ethanol as collecting solvent (marked with star, compounds of sensory relevance also appearing in olive oil sample).

Looking at the following Table 21, it is remarkable that the first extraction attempt, even having a lower distillation temperature of 100°C in the first 15min, then 130°C, has higher peak areas in the compounds Hexanal, (E)-2-Hexenal, Octanal, Nonanal and (E,E)-2,4-Heptadienal. Especially the last three compounds are characteristically for oxidation of the olive oil sample. Whilst distillation number two with higher temperature has higher peak areas in alpha-Copaene and alpha-Farnesene.

The subsequent following Figure 28 shows a graphical comparison of the extraction results illustrated in Table 21.

Table 21: Overview of extraction results using atmospheric distillation, peak areas in percent (marked in red, peak areas standing out).

	Peak areas %			
Compounds	**Olive oil**	**Ethanol**	**1. Extr.**	**2. Extr.**
Hexanal	2,75	-	0,41	0,00
Ethylbenzene	0,01	0,03	0,01	0,02
2-Methyl-4-pentenal	0,96	-	0,07	-
(Z)-3-Hexenal	4,50	-	0,06	-
1-Penten-3-ol	1,25	-	0,01	0,01
(E)-2-Hexenal	38,66	-	4,27	1,41
Octanal	0,02	-	0,13	0,00
1-Hexanol	2,00	-	0,10	-
Nonanal	0,13	0,04	2,26	0,19
(E)-2-Hexen-1-ol	2,44	-	0,23	-
Acetic acid	4,73	0,02	0,01	0,00
(E,E)-2,4-Heptadienal	0,03	-	0,15	-
alpha-Copaene	0,18	-	0,17	2,68
Benzaldehyde	0,03	-	0,02	0,01
alpha-Farnesene	0,18	-	2,90	3,93

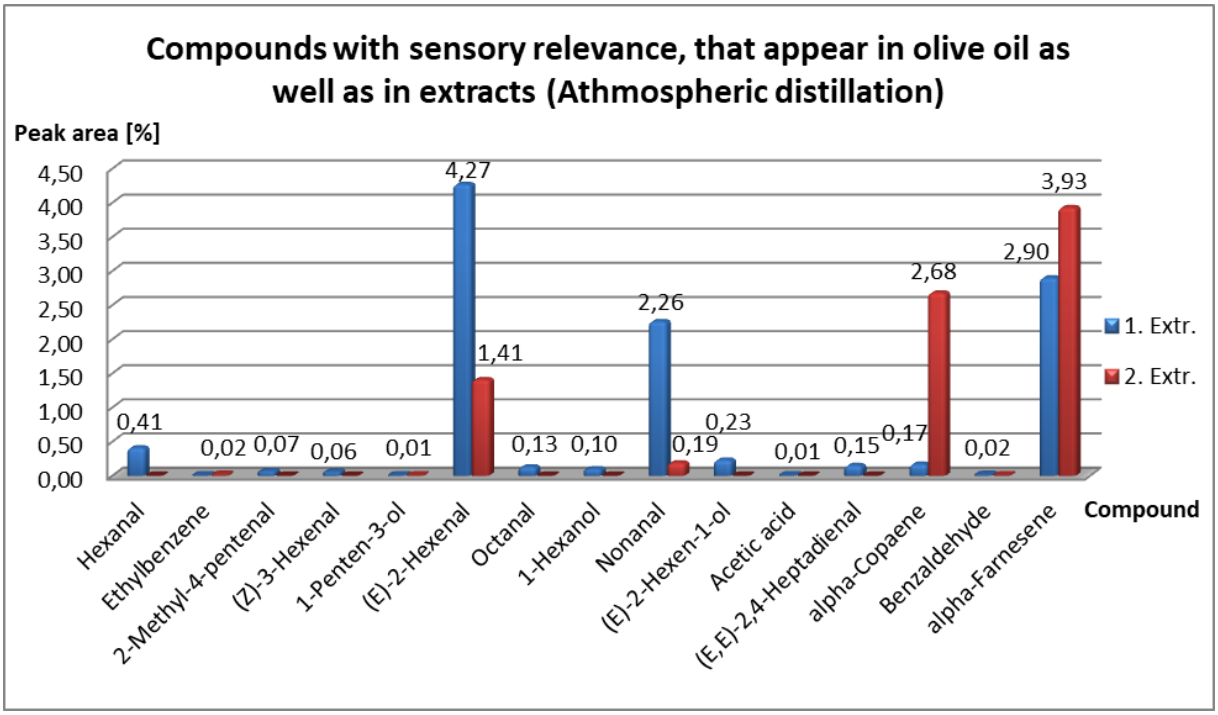


Figure 28: Graphical comparison of atmospheric distillation extraction, using 20% ethanol as collecting solvent.

3.3.3.1.2 Vacuum distillation

Figure 29 shows the GC-MS analysis results of the extracts of vacuum distillation in a rotary evaporator. Noticeable is that extract number four involving 20% ethanol and propylene glycol, mostly resembles the chromatographic profile of the propylene glycol standard.

In the distillation attempt number one, using only 20% ethanol as solvent, it was possible to obtain a larger peak of Nonanal (number six) and a larger peak of (E)-2-Hexenal (number two), after two hours of distillation. Similar to the results obtained by distillation under atmospheric conditions in Figure 26.

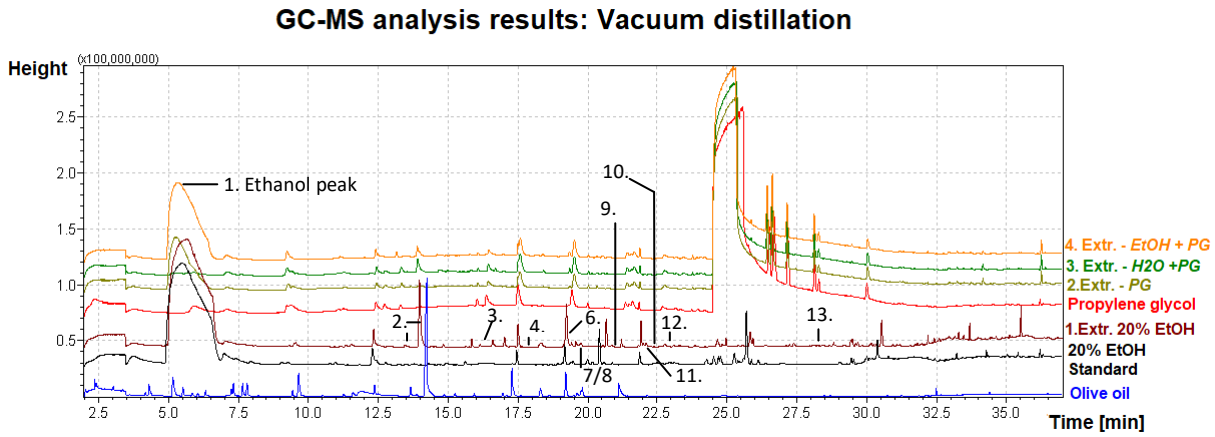
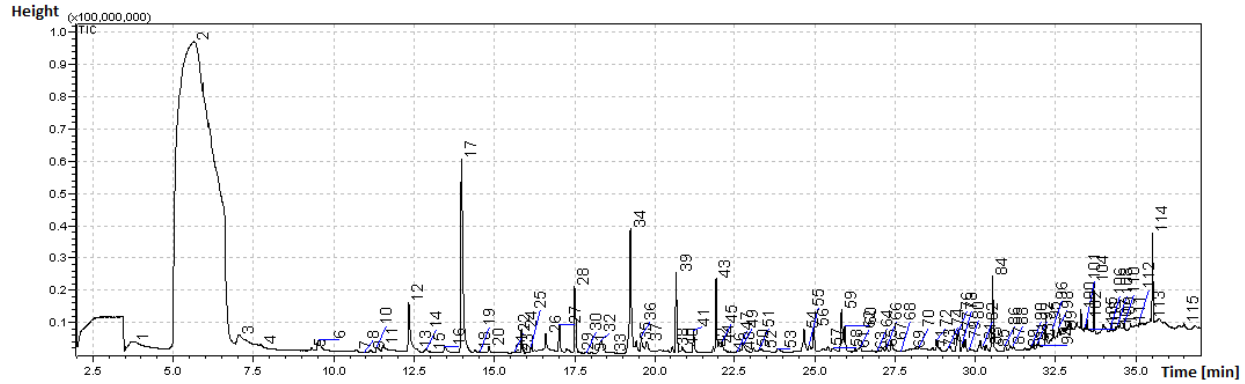


Figure 29: GC-MS analysis results of the extracts of vacuum distillation in rotary evaporator. Peaks identified with sensory relevance: 1. Ethanol, 2. (E)-2-Hexenal, 3. Octanal, 4. 6-methyl-5-Hepten-2-one, 5. 1-Hexanol 6. Nonanal, 7. 2,4-HEXADIENAL 8. (E)-2-Hexen-1-ol, 9. Acetic acid, 10. E-E-2,4-Heptadienal, 11. α-Copaene, 12. Benzaldehyde, 13. α-Farnesene.

All peaks appearing in extract number one are identified and listed below in Figure 30. Compounds marked with a star, are of sensory relevance and also appear in the olive oil sample.



Number	Compound	RT	Area %	Similarity %
1	Cyclotrisiloxane, hexamethyl-	3.758	1.05	
2		5.642	82.50	
3	Cyclotetrasiloxane, octamethyl-	7.053	0.48	
4		7.724	0.02	
5	N-Cyano-3-methylbut-2-enamine	9.303	0.03	
6	(2,2-Dimethylcyclopropyl)-methanol	9.546	0.29	
7		10.688	0.01	
8	XYLENE	10.940	0.01	
9	Benzene, 1,2-dimethyl-	11.140	0.04	
10	4-Pentenal, 2-methyl-	11.349	0.09	
11	4-Pentenal, 2-methyl-	11.532	0.14	
12	Cyclopentasiloxane, decamethyl-	12.335	0.73	
13	Benzene, 1,2-dimethyl-	12.575	0.01	
14	l-Limonene	12.878	0.02	
15	Silicic acid, diethyl bis(trimethylsilyl) ester	12.999	0.00	
16	2-Hexenal, (E)-	13.453	0.13	93
17	2-Hexenal, (E)-	13.974	3.17	95
18	1-Dodecanol (CAS) n-Dodecanol	14.398	0.03	
19	Nonane, 5-(2-methylpropyl)-	14.541	0.01	
20	1,3,6-Octatriene, 3,7-dimethyl-, (Z)-	14.827	0.07	
21		15.578	0.00	
22	Acetic acid, hexyl ester (CAS) 1-Hexyl acetate	15.642	0.01	
23	Benzene, 1,2,3-trimethyl- (CAS) 1,2,3-Trimethylbenzene	15.760	0.01	
24	Pentasiloxane, dodecamethyl-	15.844	0.21	
25	Octanal	16.134	0.06	97
26	Neryl nitrile	16.598	0.17	
27	3-Hexen-1-ol, acetate, (Z)-	17.012	0.30	
28	Malonic acid, bis(2-trimethylsilylethyl ester	17.493	0.78	
29	5-Hepten-2-one, 6-methyl-	17.634	0.01	93
30	1-Ethoxy-1-(cis-hex-3-enoxy)-ethane	17.860	0.01	
31	Cyclotetrasiloxane, octamethyl- (CAS) 1,1,3,3,5,5,7,7-OCTAMETHYL-CYCLOOCTASILOXANE	18.012	0.01	
32	1-Hexanol	18.317	0.35	95
33		18.621	0.01	
34	Nonanal (CAS) n-Nonanal	19.230	1.62	94
35	2,4-HEXADIENAL 2	19.409	0.06	96
36	2,4-HEXADIENAL 2	19.563	0.17	94
37	2-Hexen-1-ol, (E)- (CAS) trans-2-Hexen-1-ol	19.740	0.13	96
38	Decane, 1,1'-oxybis-	20.568	0.06	
39	Hexasiloxane, tetradecamethyl-	20.685	0.76	
40	Acetic acid	20.870	0.06	95
41	TRANS,TRANS-2,4-HEPTADIENAL	21.233	0.21	96
42	.alpha.-Copaene	21.842	0.05	95
43	Trisiloxane, 1,1,1,5,5,5-hexamethyl-3,3-bis[(trimethylsilyl)oxy]-	21.930	0.62	
44	TRANS,TRANS-2,4-HEPTADIENAL	22.013	0.02	94
45	Decanal	22.118	0.06	
46		22.385	0.01	
47	1-Decene, 5-methyl- (CAS) 5-METHYLDECENE	22.590	0.01	
48	3,5-Octadien-2-one	22.680	0.01	
49	Benzaldehyde (CAS) Phenylmethanal	22.773	0.10	94
50	Decane, 1,1'-oxybis-	23.099	0.05	
51		23.294	0.01	
52	1,6-Octadien-3-ol, 3,7-dimethyl-	23.350	0.01	
53	1-Octanol (CAS) Octilin	23.873	0.06	
54	Tetradecane	24.669	0.32	
55	1-Octadecanol (CAS) Stenol	24.840	0.05	
56	Heptasiloxane, hexadecamethyl-	24.972	0.18	
57	3-Hexadecene, (Z)-	25.415	0.04	
58	Heneicosane	25.586	0.04	
59	3-Octadecene, (E)-	25.836	0.43	
60	SILICATE ANION TETRAMER	25.940	0.13	
61	2-Undecene, 3-methyl-, (Z)-	26.258	0.02	
62	5-Tridecene, (Z)-	26.419	0.08	
63	Hexadecane, 2,6,10,14-tetramethyl- (CAS) Phytane	26.784	0.01	
64	2-Octylcyclopropene-1-heptanol	26.978	0.02	
65	Pentadecane	27.118	0.04	
66	2-Dodecanone	27.243	0.05	
67	Dodecanal	27.394	0.14	
68	.alpha.-Muurolene	27.698	0.01	
69	Dodecane, 4,6-dimethyl-	27.967	0.01	
70	.alpha.-Farnesene	28.256	0.02	93
71	2,4-Decadienal, (E,Z)- (CAS) trans,cis-2,4-Decadienal	28.660	0.04	
72		28.817	0.09	
73	Methyl Salicylate	28.895	0.01	
74	Cyclooctane, (1-methylpropyl)-	29.166	0.01	
75	1-Pentadecene, 2-methyl-	29.219	0.02	
76	Heptadecane	29.436	0.08	
77	SILICATE ANION TETRAMER	29.489	0.09	
78	Cyclopentane, 1-butyl-2-propyl-	29.607	0.06	
79	Cycloundecane, 1,1,2-trimethyl-	29.687	0.10	
80	Tetradecanal	29.831	0.03	
81	9-Octadecene, (E)- (CAS)	30.160	0.14	
82	2-Undecene, 9-methyl-, (E)- (CAS)	30.250	0.03	
83	1-Octadecene	30.343	0.05	
84	3-Eicosene, (E)- (CAS)	30.538	0.76	
85	3-ETHYL-PENTAN-2-OL	30.607	0.00	
86	2-Undecene, 3-methyl-, (Z)-	30.930	0.05	
87	1-Undecanol (CAS) n-Undecanol	31.087	0.04	
88	PENTAN-1,3-DIOLDIISOBUTYRATE, 2,2,4-TRIMETHYL-	31.180	0.03	
89	Eicosane	31.557	0.02	
90	Butylated Hydroxytoluene	31.743	0.03	
91	2-Nonadecanone	31.811	0.05	
92	Tetradecanal	31.927	0.03	
93	Heptasiloxane, hexadecamethyl-	31.984	0.02	
94		32.053	0.00	
95	SILICATE ANION TETRAMER	32.189	0.17	
96	3-Heptanone (CAS) Heptan-3-one	32.422	0.08	
97		32.516	0.01	
98	1-Dodecanol	32.589	0.08	
99		32.713	0.05	
100	3-Eicosene, (E)- (CAS)	33.289	0.11	
101		33.411	0.02	
102		33.470	0.07	
103	1-Dodecanol	33.572	0.01	
104	2,6-di(t-butyl)-4-hydroxy-4-methyl-2,5-cyclohexadien-1-one	33.684	0.25	
105		33.977	0.17	
106	Nonanoic acid	34.225	0.03	
107	Phenol, 5-methyl-2-(1-methylethyl)- (CAS) Thymol	34.378	0.02	
108	SILICATE ANION TETRAMER	34.427	0.02	
109	1-Hexadecanol	34.492	0.02	
110	Ethanol, 2-[2-(2-butoxyethoxy)ethoxy]-	34.631	0.04	
111	1H-Indene, 2,3-dihydro-1,1,3-trimethyl-3-phenyl-	34.836	0.03	
112	Hexadecanoic acid, ethyl ester	35.113	0.05	
113		35.455	0.01	
114	Phenol, 2,4-bis(1,1-dimethylethyl)- (CAS) 2,4-Di-tert-butylphenol	35.515	0.52	

Figure 30: GC-MS analysis results of vacuum distillation attempt number one, using 20%

ethanol as collecting solvent (marked with star, compounds of sensory relevance also appearing in olive oil sample).

Table 22 gives a general overview of the extraction results of sensory relevant compounds. The subsequent following Figure 31 shows a graphical comparison of the extraction results illustrated in Table 22.

Noticeable is that all extracts using propylene glycol (extracts 2, 3 and 4) barely trapped any compounds in comparison to distillation using only 20% ethanol. These results are similar to the ones already observed with supercritical fluid extraction.

Table 22: Overview of extraction results using atmospheric distillation, peak areas in percent (marked in red, peak areas standing out).

	Peak areas %					
Compounds	**Olive oil**	**Ethanol**	**1. Extr. EtOH**	**2. Extr. PG**	**3. Extr. H2O+PG**	**4. Extr. EtOH+PG**
Ethylbenzene	0,01	0.03	-	0,01	-	-
2-Methyl-4-pentenal	0,96	-	0,23	-	-	-
(E)-2-Hexenal	38,66	-	3,17	-	0,93	0,45
Octanal	0,02	-	0,06	-	-	-
6-Methyl-5-hepten-2-one	0,02	-	0,01	-	-	-
1-Hexanol	2,00	-	0,35	-	-	-
Nonanal	0,13	0,04	1,62	0,00	0,01	-
(Z)-3-Hexen-1-ol	5,30	-	-	-	-	0,02
2,4-Hexadienal	0,57	-	0,23	-	-	-
(E)-2-Hexen-1-ol	2,44	-	0,13	-	-	-
Acetic acid	4,73	0,02	0,06	0,01	0,01	0,01
(E,E)-2,4-Heptadienal	0,03	-	0,23	-	-	0,01
alpha-Copaene	0,18	-	0,05	-	-	-
Benzaldehyde	0,03	-	0,10	-	-	-
alpha-Farnesene	0,18	-	0,02	-	-	-
Heptanoic acid	0,01	-	0,00	0,00	0,00	-
Octanoic acid	0,04	-	0,00	0,03	0,03	0,02

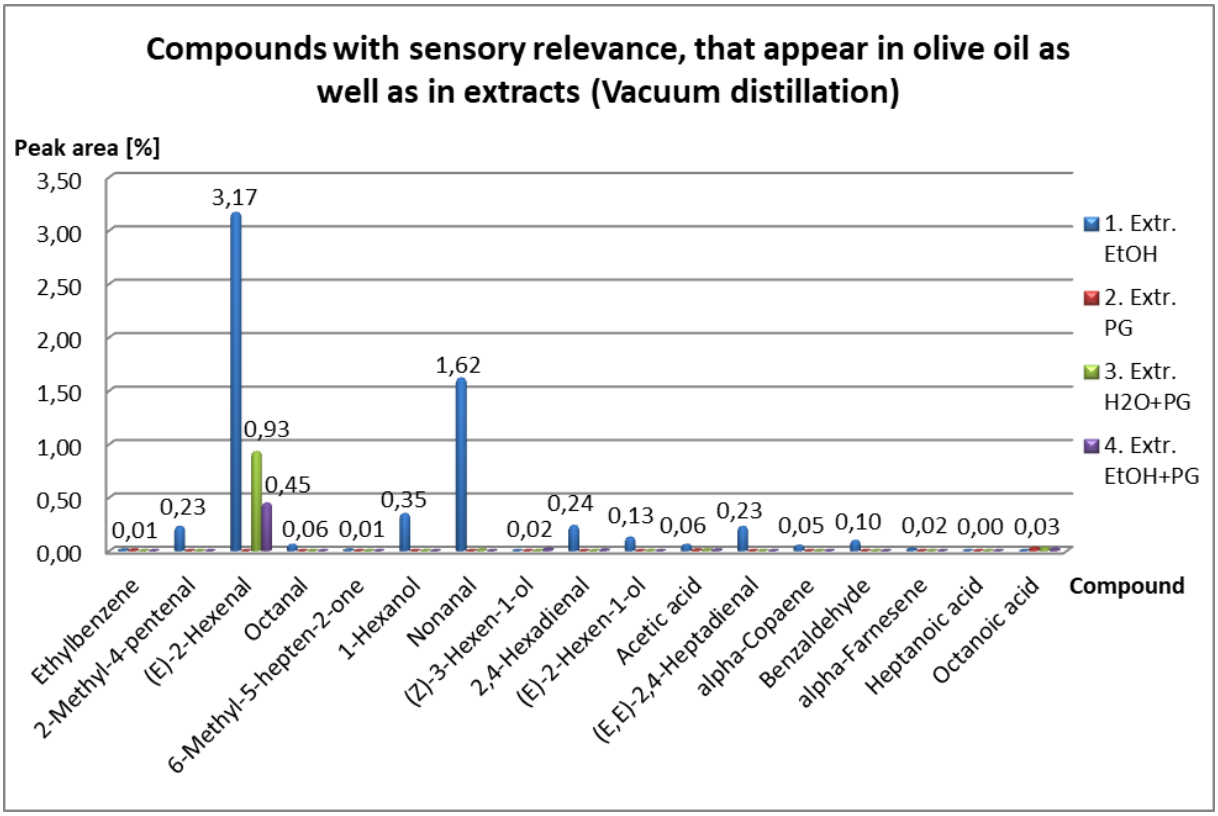


Figure 31: Graphical comparison of vacuum distillation extraction on rotary evaporator.

3.3.4 Conclusion distillation

The chromatographic results shown in Figure 31 with vacuum distillation confirm the poor collecting or trapping suitability of propylene glycol as collecting or extracting solvent for the volatile compounds already observed in Chapter 3.2.3.1.1 using supercritical fluid extraction.

Comparing distillation in atmospheric conditions to vacuum distillation, one thing that stands out between the two are the high peak areas obtained in alpha-Copaene and alpha-Farnesene with atmospheric distillation. It could be deduced that this might be in relationship with the higher temperatures applied or the higher pressure, or both in atmospheric distillation. Works from different authors applying simultaneous distillation-extraction (SDE) or hydrodistillation for the analysis of VOO aroma, reported as well a higher percentage in alpha-Farnesene [1, 39, 88, 93]. Peak areas in vacuum distillation in these two compounds were in comparison, very small.

Regarding the volatile profile of the extracts in distillation, peak areas in Nonanal and (E)-2-Hexenal were higher in atmospheric distillation, although in number of compounds it was possible to extract and trap more volatile compounds in vacuum distillation, using 20% ethanol as solvent. Despite of the milder distillation conditions in vacuum distillation, a number of

compounds characteristical for oxidation or degradation processes of the olive oil sample were identified, namely Octanal, Nonanal, 2,4-Hexadienal, (E,E)-2,4-Heptadienal, Heptanoic and Octanoic acid.

A general overview and conclusion of extraction results with SFE and distillation is given in the following chapter.

3.4 Conclusion extraction methods

Comparing SFE and distillation, looking at the obtained results from an odour and chromatographic perspective of the peak areas and number of compounds obtained by extracts, at first it appears that distillation is a more efficient approach for the extraction of volatile compounds from olive oil. Although this might as well be a controversy over the efficiency of the applied analyte trapping system for SFE. As has been mentioned before, the success of an extraction method not only depends on the extraction step itself, but also on the matrix considered as well as on the analyte trapping system [62].

The distillation results at least proved that there is more possible trapping capacity existing in 20% ethanol, than the one achieved by using SFE.

Extracted and trapped compounds such as Nonanal, Octanal, 2,4-Heptadienal, Heptanoic and Octanoic acid are known to be significantly correlated with the oxidative status of olive oil.

Hexanal amounts may be derived from either lipoxygenase action on polyunsaturated fatty acids or from chemical oxidation. This aldehyde was not only found in higher percentage in this work, applying distillation under atmospheric conditions, but also on Vichi et al. comparative study of different extraction techniques of virgin olive oil aroma, using simultaneous distillation-extraction (SDE) [39].

On the contrary, (E)-2-Hexenal, which is the most abundant volatile compound in European olive oils, is inversely related to the oxidation degree of virgin olive oil and was found to be present in lower amounts in the SFE and distillation extracts compared with the SPME analysis results of the olive oil sample.

The results obtained in this work, together with the presented literature findings indicate that the higher percentage of compounds like aldehydes and carboxylic acids observed applying distillation for extraction of volatile compounds, likely stem from oxidative alteration of the sample, rather than from a higher extraction efficiency, this was also observed in Vichi et al. work applying SDE. Alpha-Farnesene was another compound that was as well detected at a higher percentage by various authors, using SDE or hydrodistillation for the analysis of VOO aroma [1, 39, 88, 93].

The following figures give an visual overview about the best obtained collection results in the extracts by compound. Hexanal, (E)-2-Hexenal, Nonanal, alpha-Copaene and alpha-Farnesene were chosen, as other compounds were only present in trace amounts. Acetic acid was not considered, as the high amount in two extracts was only the consequence of the high amount of this compound in the used collection liquid refined oil.

The same has to be considered for the extraction results for Hexanal, the higher amounts of this compound in SFE extracts number ten and eleven are only the consequence of the presence of this compound in the refined oil. The same applies for SFE extract number nine with Nonanal, which is present in higher amounts in the flour used as support matrix for the olive oil extraction.

Noticeable again here, are the higher percentual peak areas obtained in SFE with extract number four, applying a higher pressure of 100bar with 20% ethanol as collecting solvent. 200bar in extract number three though, using propylene glycol as collecting solvent resulted in fat residuals in the extract.

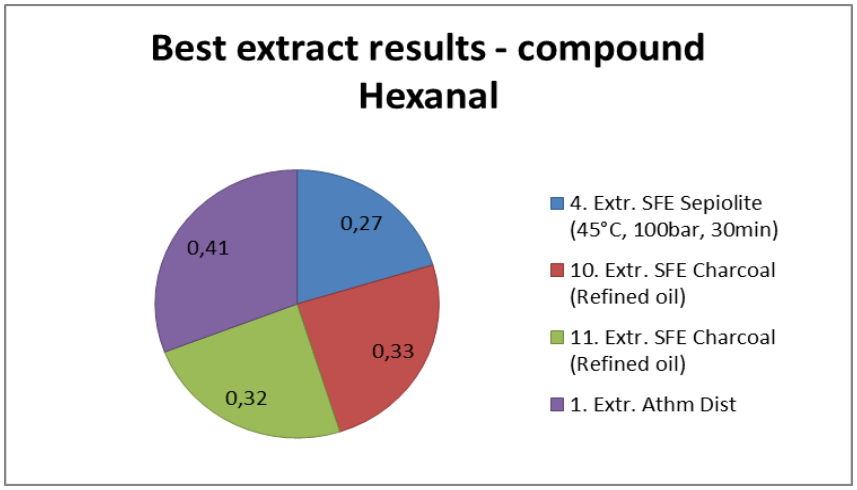


Figure 32: Best extract results by peak area % - Compound Hexanal.

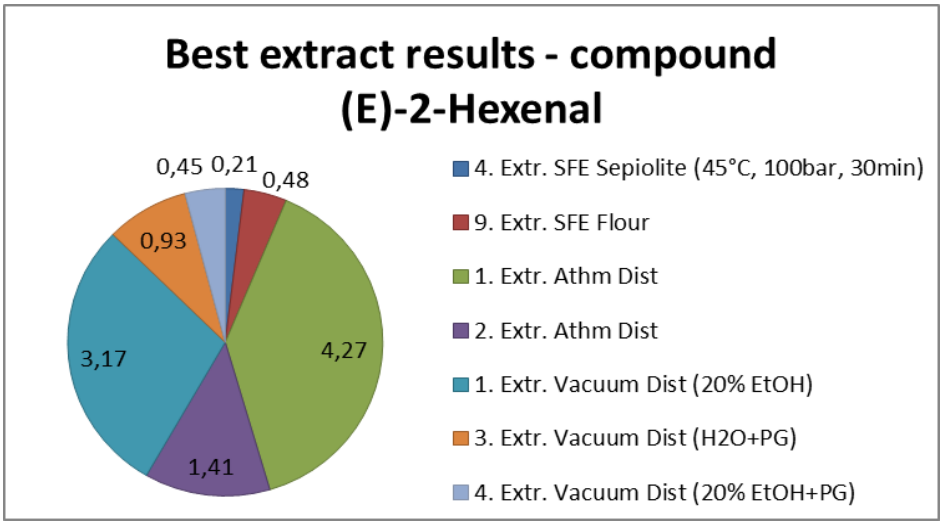


Figure 33: Best extract results by peak area % - Compound (E)-2-Hexenal.

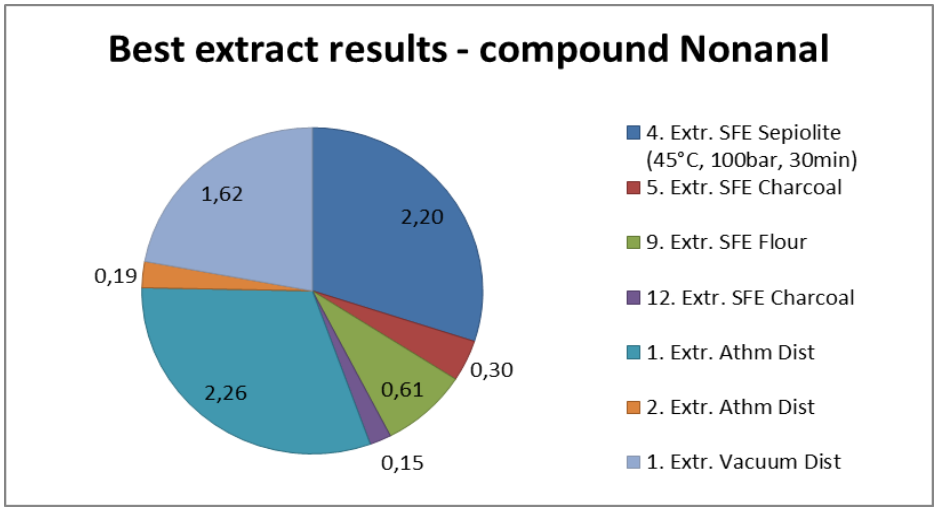


Figure 34: Best extract results by peak area % - Compound Nonanal.

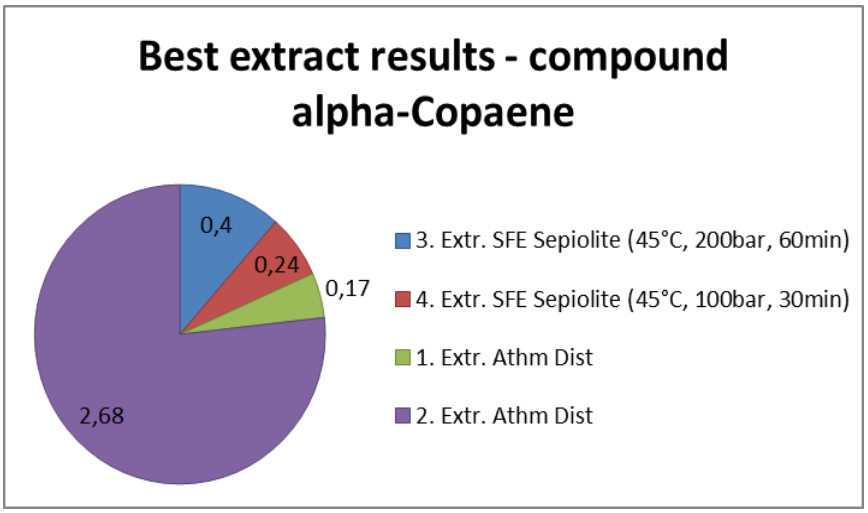


Figure 35: Best extract results by peak area % - Compound alpha-Copaene.

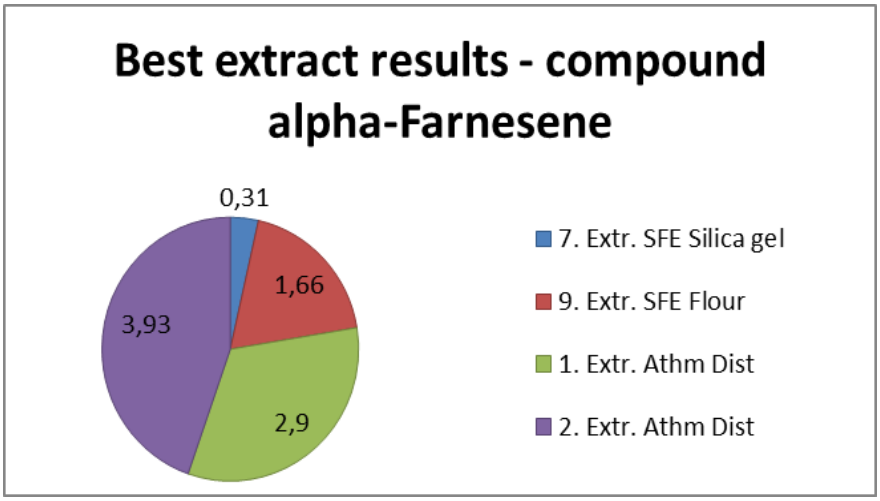


Figure 36: Best extract results by peak area % - Compound alpha-Farnesene

Concluding it is to say that the results obtained in this work show that:

- In SFE more drastic extraction conditions (higher temperature and pressure) led to the obtaining of chromatograms with peaks that showed higher areas;

- Of the collecting solvents tested, 20% ethanol proved to be the most efficient, however the extracts did not smell similar to the olive oil sample and their sensory evaluation was impaired by the smell of ethanol.

- The profile of the volatile compounds in the extracts was characterized by the presence of compounds common for oxidation of the olive oil sample.

- Distillation compared to SFE resulted in higher percentual peak areas and a higher number of extracted compounds of interest. The higher percentage of aldehydes observed in the extracts probably resulted from the oxidative alteration of the sample, rather than a higher extraction efficiency.

Considering the overall poor extraction results of the volatile compounds from the olive oil sample in the extracts, which were more characterized by compounds common for oxidation, it was possible to build two hypotheses:

1. The extraction step was not as successful as intended;
2. The conditions used for the trapping of the compounds were not the most appropriate or effective enough for trapping the target analytes. In fact, the success of an extraction method depends not only on the extraction step itself, but also on the matrix considered and the analyte trapping system [59].

D. Sarker and L. Nahar state that a “poor yield” or “poor recovery” is one of the major problems in natural products isolation, especially when the compound is present in extremely low concentration in a natural product.

To deal with this “poor-yield” issue, the authors suggested to adopt one of the following approaches:

1. Find a better source for the supply of the target compound.
2. Use genetic manipulation of the source.
3. Use semisynthesis of the target compound from a more abundant precursor.
4. Perform total synthesis of the target compound.
5. Utilize tissue or cell culture production [70].

More viable for the target compounds of olive oil volatile compounds in this case seem to be option one, three, four or five, although option number five might be associated to more expenditure and unnecessary effort, as most of the in the olive oil identified compounds are already purchasable as reference standards [94].

4 Conclusion and considerations

The aim of this work was to contribute through extraction methods to the preparation of odour standards corresponding to the defects and characteristic scents, prepared out of typical and atypical olive oil samples.

For this purpose, in a first step 34 different olive oil samples with and without sensory defects, from different regions in Portugal were analysed by HS-SPME with GC-MS.

Volatile compounds which are responsible for the olive oils characteristic aroma, present in the samples were identified based on literature descriptions. In a second step, adequate extraction methods for the extraction of the volatile compounds were tested.

HS-SPME in combination with GC-MS enabled to identify in average 172 volatile compounds of which in average 51 per sample could be identified through literature research being of sensory relevance and establish an association to their respective sensory attributes and the olive oils volatile profile.

PCA results allowed the separation of samples with sensory defect of the ones presenting no sensory defect. As the defective samples were strongly characterized by higher values of compounds related to rancid olive oil and markers related to off-flavours of virgin olive oil oxidation.

These results laid the foundation for the continuous work as the results are important to evaluate in a next step the extraction efficiency and to determine the success of the applied extraction methods and to evaluate the extraction conditions used in order to obtain high quality odour standards to be used in the training and monitoring of sensory analysis panels.

After the selection and the identification of the envisaged target extracts/compounds it is then necessary to define and to choose the most efficient methodologies to be employed in the extraction process.

The extraction of volatile compounds is a complex process that is influenced by several factors. Depending on the objective of the extraction, different process techniques and extraction conditions might be used [56].

Extracting the compounds of interest from the matrix in which they are embedded needed several issues to be taken into account. These include the polarity and volatility of the extractives, the state of matter of the raw material to be extracted, the used solvent, the process temperature, the applied pressure and time are the main variables involved in most extractions and they are irrevocably associated with the success of the process.

It is quite rare to find a specific extraction method and a solvent mixture that presents a high and specific selectivity for the main target compounds and that will lead to high purity ex-

tracts [56]. As there is no single perfect method for extraction, purification and isolation of compounds [95].

The success of an extraction method not only depends on the extraction step itself but also on the matrix considered as well as on the analyte trapping system.

Quantitative extraction conditions cannot be developed and evaluated unless the collection step is efficient. Thus, the first task of the analyst is to optimize the collection system and determine its efficiency for the target analytes [62].

For the extraction of the odour compounds in this work, distillation and Supercritical fluid extraction (SFE) were used, due to availability in the laboratory and compatibility with the works objective.

An in depth literature research about the extraction of volatile compounds from olive oil samples showed that most work done with olive oil aim for the extraction of the VOCs for subsequent analysis in gas chromatography, employing often the use of health hazardous solvents. No similar work in the literature with the aim to use these extracts to obtain sensory reference standards was found.

The results obtained through extraction showed:

- In SFE more drastic extraction conditions (higher temperature and pressure) led to the obtaining of chromatograms with peaks that showed higher areas;

- Of the collecting solvents tested, 20% ethanol proved to be the most efficient, however the extracts did not smell similar to an olive oil sample and their sensory evaluation was impaired by the smell of ethanol.

- The profile of the volatile compounds in the extracts was characterized by the presence of compounds common for oxidation of the olive oil sample.

- Distillation compared to SFE resulted in higher percentual peak areas and a higher number of extracted compounds of interest. The higher percentage of aldehydes observed in the extracts probably resulted from the oxidative alteration of the sample, rather than a higher extraction efficiency.

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1. Find a better source for the supply of the target compound.
2. Use genetic manipulation of the source.
3. Use semisynthesis of the target compound from a more abundant precursor.
4. Perform total synthesis of the target compound.
5. Utilize tissue or cell culture production [70].

More viable for the target compounds of olive oil volatile compounds in this case seem to be option one, three, four or five, although option number five might be associated to more expenditure and unnecessary effort, as most of the in the olive oil identified compounds are already purchasable as reference standards [94].

Pressurised hot water extraction might be a viable alternative to conventional extraction techniques (Soxhlet and/or standard solvent extraction procedures). There is evidence suggesting that it may compete favourably with more recent extraction techniques such as SFE.

Extraction of flavourings from natural sources with subcritical water demonstrated its ability to selectively extract different classes of compounds, the more polar organics being extracted at lower temperatures and the less polar ones at higher temperatures. Therefore, the selectivity of subcritical water extraction (a preference for more polar organics at milder conditions) is contrary to that of supercritical CO_2 (preferring non-polar over polar organics) [85].

This might be of advantage for the extraction of the volatile compounds from olive oil, which showed to be more polar.

Today, the defining techniques for producing high quality flavours are extraction, distillation, fermentation and chemical synthesis [96]. Flath, Forrey and Guadagni almost 50 years ago tried a different approach for attaining olive oil aroma imitations. They added selected components likely to contribute to an olive oil like aroma to an odourless oil base for comparison with authentic olive oil samples. In their trials one mixture had an aroma approaching that of olive oil, but still lacked certain components needed to provide the fruitiness characteristics of a quality olive oil [24].

In conclusion it is to say, that today the volatile composition and the relationship between volatile compounds and their sensory contribution to the overall olive oil flavour seem to be well explored. Despite of substantial developments of extraction and separation techniques, the isolation of natural products from raw materials leading to high yields, employing safe and non-toxic solvents or solvent-mixtures, without the compounds degradation or loss remains still a challenging task.

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