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Carina Filipa Pedrosa da Costa

Volatile exometabolome analysis of *Aspergillus niger* and search for molecular biomarkers pattern

Análise do exometaboloma volátil de *Aspergillus niger* e pesquisa de um padrão de biomarcadores moleculares

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Dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Biologia Aplicada, ramo de Microbiologia Clínica e Ambiental, realizada sob a orientação científica da Professora Doutora Sílvia Maria da Rocha Simões Carriço, Professora Auxiliar do Departamento de Química da Universidade de Aveiro, e coorientação da Professora Doutora Maria Adelaide de Pinho Almeida, Professora Auxiliar do Departamento de Biologia da Universidade de Aveiro.

Dedico este trabalho aos meus pais.

“O êxito da vida não se mede pelo caminho que você conquistou,
mas sim pelas dificuldades que se superou no caminho”

o júri

Presidente

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palavras-chave

Aspergillus niger, infeções fúngicas, exometaboloma, HS-SPME/GC×GC–ToFMS, biomarcadores moleculares voláteis

resumo

As infeções fúngicas têm aumentado bastante em populações de risco, nomeadamente em pacientes imunocomprometidos, provavelmente devido a atrasos no diagnóstico das infeções fúngicas. A metabolómica microbiana surge como um poderoso recurso de triagem dos metabolitos produzidos por microrganismos. Esta fornece informações sobre o estado de organismos biológicos, que podem ser usados como uma ferramenta de diagnóstico para infeções fúngicas através de um padrão de metabolitos fúngicos. Assim, este trabalho teve como objetivo estudar em profundidade o exometaboloma de *Aspergillus niger*, a fim de estabelecer um padrão metobolómico alvo que caracterize o *A. niger*. Foi usada uma metodologia baseada em microextração em fase sólida no espaço de cabeça combinada com cromatografia de gás bidimensional abrangente acoplada a espectrometria de massa por tempo de voo (HS-SPME / GC×GC-ToFMS). O exometaboloma de *A. niger* foi analisado em diferentes condições de crescimento: temperatura (25 e 37 °C), tempo de incubação (3 e 5 dias) e meio de cultura (meio sólido e líquido). O exometaboloma do *A. niger* incluiu 430 metabolitos, distribuídos em várias famílias químicas, sendo os mais importantes os álcoois, aldeídos, ésteres, cetonas, hidrocarbonetos e terpenos. Observaram-se diferenças entre os metabolitos voláteis produzidos em diferentes condições de crescimento, sendo a maior abundância relativa determinada para os 5 dias de crescimento, a 25 °C, utilizando meio sólido. Estes resultados indicaram a alta complexidade do exometaboloma do *A. niger*. Um subconjunto de 44 metabolitos, que estavam presentes em todas as condições de crescimento testadas, foi definido como um padrão metabolómico alvo para o *A. niger*. Este padrão pode ser usado na deteção de infeções fúngicas por esta espécie e ser futuramente explorado para diagnóstico de infeções fúngicas. Além disso, este subconjunto de metabolitos foi comparado com amostras de *Candida albicans* (levedura) e *Penicillium chrysogenum* (fungo filamentoso), e a análise discriminante com método dos mínimos quadrados parciais (PLS-DA) foi aplicada. Os resultados mostraram claramente que este subconjunto de metabolitos permitiu distinguir estes microrganismos. Para validar o modelo do PLS-DA, o teste das permutações foi aplicado, e um modelo estatísticamente significante para os 44 metabolitos foi obtido com uma capacidade preditiva Q^2 de 0.70 para o *A. niger*. Quando o subconjunto de compostos foi reduzido para 16 (obtidos pelo parâmetro Importância da Variável na Projeção (VIP)), o modelo obtido teve uma capacidade preditiva Q^2 de 0.86 para o *A. niger*, que foi significantemente superior, sendo mais robusto que o anterior. A diminuição de 44 para 16 metabolitos, reduziu o tempo de análise necessário e as condições utilizadas foram semelhantes às condições utilizadas em contexto clínico, (meio sólido e 25 °C e aproximadamente 1 semana). No entanto, neste estudo, foi possível reduzir o tempo para 3 dias. Em conclusão, estes 44 biomarcadores moleculares voláteis poderão ser úteis para o diagnóstico de infeções fúngicas, e podem ser explorados em contexto clínico.

keywords

Aspergillus niger, fungal infections, volatile exometabolome, HS-SPME/GC×GC–ToFMS, volatile molecular biomarkers

abstract

Fungal infections have greatly increased in risk populations, namely in immunocompromised patients, probabily because the diagnosis of fungal infections is delayed. Microbial metabolomics arises as a powerful feature screening the metabolites produced by microorganisms. It provides information regarding the state of biological organisms which can be used as a diagnostic tool for diseases through fungal metabolites pattern. Thus, this research aimed to in-depth study of the *Aspergillus niger* exometabolome, in order to establish a targeted metabolomic pattern that characterizes *A. niger*. A methodology based on headspace-solid phase microextraction combined with comprehensive two-dimensional gas chromatography coupled to mass spectrometry with a high resolution time of flight analyser (HS-SPME/GC×GC-ToFMS) was used. *A. niger* exometabolome was analysed in different growth conditions: temperature (25 and 37 °C), incubation time (3 and 5 days), and culture medium (solid and liquid medium). *A. niger* exometabolome included 430 metabolites, distributed over several chemical families, being the major ones alcohols, aldehydes, esters, hydrocarbons, ketones and terpenoids. Differences among volatile metabolites produced under different growth conditions were observed, being the major relative abundance determined for 5 days of growth, at 25 °C, using solid medium. These results indicated the high complexity of *A. niger* exometabolome.

A subset of 44 metabolites, which were present in all previously tested growth conditions, was defined as the *A. niger* targeted metabolomic pattern. This pattern may be used in detection of fungal infections by this specie and be further exploited to fungal infections diagnosis. Furthermore, this subset of metabolites was compared with samples of *Candida albicans* (yeast) and *Penicillium chrysogenum* (filamentous fungi), and Partial Least Squares Discriminant Analysis (PLS-DA) was applied. The results clearly showed that this metabolites subset allowed the distinction between these microorganisms. In order to validate the PLS-DA model, permutation test was applied, and a statistically significant model for 44 metabolites was obtained with a predictive Q^2 capability of 0.70 for *A. niger*. When the subset of compounds were reduced to 16 (obtained by Variables Importance in Projection (VIP) parameter), the obtained model had a predictive Q^2 capability of 0.86 for *A. niger*, which was significantly higher, being more robust than the previous. The decrease of 44 to 16 metabolites, reduced the require analysis time and the conditions used were similar to the conditions used in clinical context, (solid medium, at 25 °C and *ca.* 1 week). However, in this study was possible to reduce the time for 3 days. In conclusion, these 44 volatile molecular biomarkers could be useful for diagnosis of fungal infections, and they can even be further exploited in clinical context.

Index

List of acronyms and abbreviations

a.u.	Arbitrary units
ANOVA	Analysis of variance
CFU	Colony forming-units
CFU mL^{-1}	Colony forming-units per millilitre
1D-GC	One-dimensional gas chromatography
DVB/CAR/PDMS	Divinylbenzene/carboxen/poly(dimethylsiloxane)
GC×GC	Comprehensive two-dimensional gas chromatography
GC×GC – ToFMS	Comprehensive two-dimensional gas chromatography coupled to mass spectrometry with a high resolution time of flight analyser
GC-MS	Gas chromatography-mass spectrometry
HS	Headspace
IA	Invasive aspergillosis
IFI	Invasive fungal infections
MALDI-TOF MS	Matrix-assisted laser desorption ionization time-of-flight mass spectrometry
Min	Minutes
MS	Mass spectrometry
m/z	Mass to charge ratio
NMR	Nuclear magnetic resonance
p	*p*-value
PCA	Principal Component Analysis
PLS-DA	Partial Least Squares Discriminant Analysis
RI	Retention index
s	Seconds
S/N threshold	Signal-to-noise threshold
SPME	Solid phase microextraction
TIC	Total ion chromatograms
ToFMS	Time-of-flight mass spectrometry
t_R	Retention time
VIP	Variables Importance in Projection
VOC	Volatile organic compounds
YGC	Yeast Glucose Chloramphenicol Agar

Chapter 1: Introduction

1.1. Fungi

Fungi are organisms that can occur in two basic forms: yeasts and filamentous fungi. Yeasts are single and small colonies, whereas filamentous fungi occur in hyphae form. Some fungi are dimorphic, existing as either as yeasts or filamentous fungi depending on the external environmental factors such as temperature (Garber, 2001). Fungi are eukaryotic microorganisms of clinical, environmental and food areas relevance (Wang, *et al.*, 2005). Fungi are aerobic, i.e, grow in the presence of oxygen, and grow at low pH values (Madigan, *et al.*, 2012). These organisms are ubiquitous in the environment spreading in their natural environments with no need for human or animal substrates (Garber, 2001). The human activity can be the cause for dispersing fungal diseases and modifying natural environments. Emerging fungal infections will have vast implications for human health and ecosystems, unless measures to strengthen biosecurity worldwide are taken (Fisher, *et al.*, 2012).

In the last years the number of fungal pathogenic species has significantly increased. The main reason is that population at risk has been growing, namely immunosuppressive patient population. Consequently, the incidence of invasive fungal infections have increased, leading to advances in diagnostic tools (Oz & Kiraz, 2011, Alastruey-Izquierdo, *et al.*, 2013) In general, the bacterial infections are more frequent than fungal infections but the mortality rates by fungal infections are significantly higher than bacterial ones (Oz & Kiraz, 2011).

1.2.*Aspergillus niger*

Aspergillus genus is represented by filamentous fungi in *Ascomycota* phylum. The sexual spores are produced endogenously in a saclike ascus typically produced in the well-differentiated ascocarp. It also has differentiated structures of septate hyphae and radiates chains of conidiospores (conidia) (Pelczar, *et al.*, 1993, Pan, *et al.*, 2011).

Aspergillus spp. can cause opportunist infections in immunocompromised hosts, do not cause invasive infections in healthy host. *Aspergillus niger* is a filamentous fungi (Figure 1) that causes opportunist fungal infections which has increased in the last years both in pediatric patients as adults (Spicer, 2000, Fernandez, *et al.*, 2014).

Aspergillus niger, as well as other *Aspergillus* species produces a mycotoxin denominated ochratoxin A. This fungus produces dark pigmented roughened spores

originating mature colonies within two or six days. Its growth begins with a yellow colony that soon develops into a black one (Forbes, *et al.*, 2007).

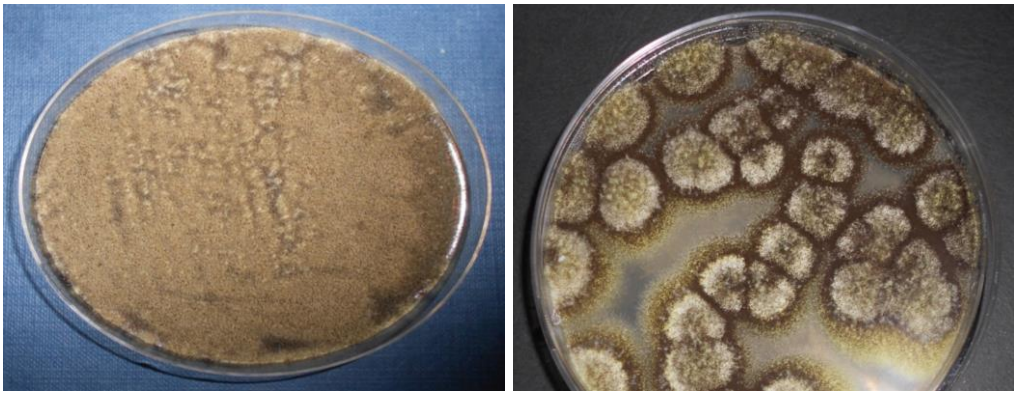

Figure 1 – Filamentous fungus - *Aspergillus niger*.

1.3. Fungal infections

Fungal infections can be classified according to the affected tissues and organs as superficial (cutaneous and subcutaneous) or systemic (Madigan, *et al.*, 2012). Superficial infections are characterized by infecting the outermost layer of the skin. The cutaneous infections affect the skin epidermis, hair and nails, causing a host response, whereas subcutaneous involve inner most layers, the dermis, making the treatment more difficult. The systemic infections are the most serious category of fungal infections. These infections are caused by yeasts or filamentous fungi invading the bloodstream or visceral regions, and entails fungal growth in internal organs of the body (Madigan, *et al.*, 2012).

Invasive fungal infections (IFI) have acquired a great clinical importance because of an increase in the risk populations. In general, this population includes patients with HIV infection, transplant recipients, cancer patients and other individuals receiving immunosuppressive treatments (Badiee, *et al.*, 2009, Karageorgopoulos, *et al.*, 2011, Wessolossky, *et al.*, 2013). The number of patients that survive with IFI depends on early diagnosis, however the clinical manifestations of these infections are nonspecific (Badiee, *et al.*, 2009). Some risk factors include immunosuppression and modifications in mechanisms of the skin, such as burn wounds and surgical wounds (McPherson & Pincus, 2011).

The reduced ventilation in buildings for improving its isolation has generated environments that favour the growth of filamentous fungi (Meheust, *et al.*, 2014). Several

studies have shown that it is very important to have air-control measures for reducing dissemination of airborne biological particles in buildings, namely in hospitals (Jean Pierre Gangneux, *et al.*, 2006).

Nosocomial infections can be defined as infections caused by pathogens originating within the hospital facilities, progressively developing resistance to antifungal drugs (Haddad, *et al.*, 2004). These infections may have various sources namely hospital personnel, air, devices (drains, implants and catheters), prolonged stay in the intensive care units, prolonged neutropenia, immunosuppression, solid organ transplant, diabetes mellitus, chronic renal failure and severe burns (Dhillon & Clark, 2011).

Extensive burns represent a serious problem, being one of the most traumatic experiences that an individual can suffer. Directly or indirectly, the fungi reach all compartments and systems of the body (Hettiaratchy & Dziewulski, 2004, Rempel, *et al.*, 2011). Burns may be defined as losses caused by the coating body energy transfer from multiple agents (Rempel, *et al.*, 2011). The infection is always a threat to the life of the burnt patient since the exposure to infectious agents is not controllable (Madigan, *et al.*, 2012). The burnt patient, after losing the protection of the skin, which is the first line of defence against invading microorganisms, presents a substantially increased risk of contracting infection (Bagdonas, *et al.*, 2004, Wibbenmeyer, *et al.*, 2006).

The most common pathogens causing fungal infections are *Aspergillus* spp. and *Candida* spp., which can cause invasive aspergillosis (IA) and candidemia, respectively. However *Cryptococcus neoformans* and agents of Mucormycosis (*Rhizopus*, *Mucor* and *Absidia* genus) also cause infections (Sipsas & Kontoyiannis, 2012, Kaul & Chauhan, 2014). In the last decade, some fungal infections have been increasing, such as candidemia and invasive aspergillosis (Figure 2). Actually this type of fungal infections contributes for the death rate. Otherwise, crytococcosis, mucomycosis and *Pneumosystis* pneumonia had less cases incidence of in the population, which tend to be less threatening to humans (Bitar D, *et al.*, 2014).

Aspergillus species can cause invasive aspergillosis that is a major cause of morbidity and mortality in immunocompromised patients (Dinand, *et al.*, 2013, Ergene, *et al.*, 2013). Invasive aspergillosis appears to be an opportunistic infection in critically unhealthy patients that can be caused by different *Aspergillus* spp. and can implicate multiple organs. In this infection, the mortality rate is high, which strongly suggests the

need for prevention or earlier diagnosis and treatment (Badiee, *et al.*, 2011). These fungi invade lung tissue through the respiratory tract, enter in the bloodstream, and can disseminate to other organs (Pan, *et al.*, 2011).

Candida albicans is an opportunistic pathogen which causes infections in immunocompromised individuals or in altered immune system. It is a dimorphic fungus that is capable to grow in distinct morphologies (yeast and pseudohyphae) which confer advantages in the course of infection (Han, *et al.*, 2011, Kim & Sudbery, 2011). Candidiasis and candidemia have increased in last years, particularly in intensive care units (Sipsas & Kontoyiannis, 2012)

Penicillium genus represents filamentous fungi from *Ascomycota* phylum (Black, 2008). *Penicillium* spp. are an opportunistic fungi that can cause fatal diseases. On the other hand, these fungi play a significant role in the medical community, as they can produce antibiotics like penicillin, responsible to inhibit the growth of some bacterial cultures. *Penicillium chrysogenum* has an important role in human life in various forms as a pathogen and allergen (Tortora, *et al.*, 2013).

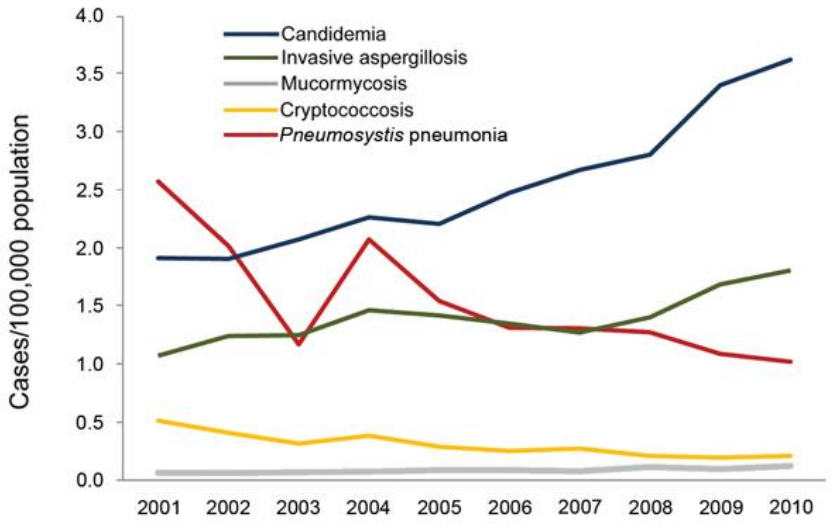

Figure 2 - Trends in the incidence of several fungal infections (candidemia, invasive aspergillosis mucormycosis, cryptococcosis and *Pneumocystosis* pneumonia) from 2001 to 2010 in France (Bitar D, *et al.*, 2014).

1.4. Diagnosis of fungal infections

The optimization of a rapid diagnosis method for fungal infections is very important given the increasing number of opportunistic fungal infections in humans, mainly due to factors such as the ability of microorganisms grow at 37 °C at low pH and their capacity to produce toxins (Forbes, *et al.*, 2007). Furthermore, many patients with clinical suspicion for the presence of fungal infection are treated empirically with antifungals that may involve the unnecessary use of potentially toxic and costly drugs (Karageorgopoulos, *et al.*, 2011).

The diagnosis of invasive infections involves several conventional methods, such as microscopy, histology and cellular culture. However these methods are time consuming and their efficiency is low (Erjavec & Verweij, 2002). In alternative, serological (detection of antibodies and antigens) and molecular methods (polymerase chain reaction - PCR) can be used (Badiee, *et al.*, 2009, Karageorgopoulos, *et al.*, 2011). New methods for diagnosis of fungal infections have been developed, such as, matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) and ergosterol detection (Croxatto, *et al.*, 2012, Meheust, *et al.*, 2014).

In conventional methods, the total identification time including culturing time is of approximately 2-14 days, depending on different growth rate from different species (Pan, *et al.*, 2011). The use of microscopy makes the identification of the fungal spores often difficult because only a small number of fungal spore types can be assuredly identified, and some spores cannot be differentiated because some are highly similar, for example *Aspergillus* spp. and *Penicillium* spp. (Meheust, *et al.*, 2014). Culture-based methods can give information about the quantitative and qualitative data on viable and culturable fungi. However, the results are influenced by factors such as culture medium, temperature and competition between fungi species (Meheust, *et al.*, 2014). These methods are cheap but require at least a seven day incubation period. It has the disadvantage of not detecting non-viable and non-culturable fungi (Meheust, *et al.*, 2014). They are time consuming and frequently lead to false species identification (Pan, *et al.*, 2011). The disadvantage of conventional methods is the inability to definitely distinguish filamentous fungi, which may compromise diagnostic accuracy and the estimates of therapeutic efficacy in patients (Hope, *et al.*, 2005).

The immunological methods consist in the antigen detection, such as galactomannan and (1-3)-β-D-glucan. The galactomannan is a cell wall polysaccharide, released by growing hyphae of *Aspergillus* species and others fungi. This component can indicate tissue invasion in clinical situations (Schuetz, 2013). Some studies use the Platelia kit *Aspergillus* antigen immunoassay produced by Bio-Rad Laboratories (Cummings, *et al.*, 2007). Another serum marker for the presence of IFI is (1-3)-β-D-glucan, a cell wall component of most fungi that can be detectable in blood during most of the invasive fungal infections (Alexander, 2002, Badiee, *et al.*, 2009, Karageorgopoulos, *et al.*, 2011).

The molecular methods allow to detect fungal infections with a high sensibility (Meheust, *et al.*, 2014). Examples of these methods are polymerase chain reaction (PCR) amplification and DNA sequence analysis to detect and identify microorganisms. PCR has some disadvantages, such as the contamination of samples during analysis (Meheust, *et al.*, 2014).

Until recent times, the development of other techniques has revolutionized the routine identification of microorganisms in clinical laboratories. The technology matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) has been adapted to the constraint of clinical diagnosis laboratories and has replaced and/or complemented conventional identification techniques (Croxatto, *et al.*, 2012). This technology generates characteristic mass spectral fingerprints that are unique signatures for each microorganism and are thus ideal for an accurate microbial identification having a potential to be used for strain typing and identification. Some studies showed that MALDI-TOF MS has been used to characterize a variety of microorganisms (Croxatto, *et al.*, 2012). It represents a strong challenge to microscopic and molecular methods (Chalupová, *et al.*, 2014).

For fungi identification, ergosterol can be used. It is generally analysed in many studies as an index of fungal biomass (Meheust, *et al.*, 2014). Ergosterol is the major sterol found in fungal cell membrane and has been suggested for the determination of fungal contamination (Kavanagh, 2011, Porep, *et al.*, 2014). Ergosterol content can be used to estimate the fungal biomass in different environments, for example, in the soil or in the aquatic environments. However, the amount of ergosterol in fungal tissue is not constant, but there is a strong correlation between ergosterol and fungal dry mass (Parsi & Gorecki, 2006). The concentration of ergosterol depends on the fungal species, age of culture,

development stage (hyphal formation or sporulation and growth phase) and growth conditions (growth media, pH and temperature) (Parsi & Gorecki, 2006). It can be considered as a fungal biomarker because it is specific of fungi (Parsi & Gorecki, 2006).

1.5. Microbial metabolomics approach

1.5.1. Concept

Metabolomics is the comprehensive study of the metabolites (low molecular weight molecules) of chemical processes, such as metabolites of cells and tissues of an organism under a given set of conditions, aimed at the identification of the metabolome of a biological system (Gowda, *et al.*, 2008, Tang, 2011, Gahlaut, *et al.*, 2013). The metabolome is the set of all metabolites present in cells that are participants in general metabolic reactions, essential for the growth, maintenance and normal function of a cell (Dunn & Ellis, 2005). These metabolites are the end products of cellular processes in biological cell, tissue, organ or organism (Gahlaut, *et al.*, 2013). Metabolome is made up of two parts, the endometabolome (intracellular metabolites) and the exometabolome (extracellular metabolites) (Nielsen & Jewett, 2007). Metabolomics exhibits a critical role in environmental interactions, functional genomics, secondary metabolism and metabolic profiles (Tang, 2011, Gahlaut, *et al.*, 2013).

Microbial metabolomics constitutes an integrated component of systems biology, measures metabolites in order to facilitate the understanding of microbial interactions, cellular functions and dynamics of cell metabolism (Tang, 2011, Liebeke & Lalk, 2014). Microorganisms are ideal for conducting systems biology studies because they are easy to manipulate and have an important role in human health, as well environment (Tang, 2011). Through the study of the complete set of microorganism metabolites and the monitoring of the global outcome of interactions between their development processes, the metabolomics can provide accurate information about the physiological state of the cell (Tang, 2011). The main challenge of metabolomics is the ability to identify and quantify all intracellular and extracellular metabolites present in a specific sample. The number of these metabolites varies among different organisms, from hundred to hundreds of thousands (Tang, 2011). Metabolites have different chemical properties, such as weigh, polarity and solubility, and in physical properties, like volatility (Dunn & Ellis, 2005). Metabolomics can measure

many different type of compounds, including primary metabolites, secondary metabolites and quorum sensing compounds (Liebeke & Lalk, 2014). The basis of the metabolomics has as instrumental tool the mass spectrometry (MS) and nuclear magnetic resonance (NMR) (Zhang, *et al.*, 2012).

The analysis of metabolites in tissues or body fluids has become important to monitoring the state of biological organisms and can be used as a tool for diagnostic of diseases (Zhang, *et al.*, 2012). Metabolomics can have different applications namely determination of metabolic biomarkers that change as an indicator of the presence of a disease, bacterial characterization and determination of the biochemical or environmental stress effect in microorganisms (Dunn & Ellis, 2005).

Filamentous fungi produce a broad range of secondary metabolites (Calvo, *et al.*, 2002, Roze, *et al.*, 2010). Table S1 (Supplementary Information) summarizes the metabolites produced by different fungal species, namely, *Aspergillus* spp, *Mucor* spp., *Penicillium* spp., *Saccharomyces cerevisiae* and other fungi. 70% of species produces 3-methyl-1-butanol and more than 50% of species produces 1-octen-3-ol and limonene. 50% of species produces 3-octanone, 40% produces 2-methyl-1-butanol and 35% of species produces 2-methyl-1-propanol and 3-octanol.

These compounds are derived from others compounds formed during primary metabolism, for example amino acids, nucleotides, carbohydrates or acyl-CoA (Roze, et al., 2010).

Metabolic pathways of fungi can be lead to the production of volatile organic compounds (VOC), for instance several filamentous fungi metabolize alcohols, like ethanol which can be oxidized to acetate via acetaldehyde and thereby enter in various intermediate metabolic pathways (Keller & Hohn, 1997).

In fungi, mitochondria are an important organelle. During glucose respiration under aerobic conditions, pyruvate is formed through glycolysis, and enters in the mitochondria, where is oxidatively decarboxylated to acetyl-CoA by pyruvate dehydrogenase, which acts as the link between glycolysis and the cyclic series of enzyme catalysed reactions known as the tricarboxylic acid (TCA) cycle. This cycle represents the common pathway for the oxidation of sugars, the carbon sources for filamentous fungi (Kavanagh, 2011). Glycolysis is a key pathway of metabolism.

1.5.2. Metabolomic profile of *Aspergillus niger*

According to the literature available there are reported various metabolites that are produced by *Aspergillus niger* which are distributed over several chemical families, such as: alcohols, aldehydes, esters, ethers, furan-type compounds, ketones, hydrocarbons, monoterpenes and sesquiterpenes. Specifically these reported compounds are ethanol; 1-propanol; 2-methyl-1-propanol; 2-methyl-1-butanol; 3-methyl-1-butanol; 2-pentanol; 1-octen-3-ol; 3-octanol; acetaldehyde; ethyl propanoate; ethyl butanoate; ethyl tiglate; 3-methyl-2-butenoic acid ethyl ester; iso-amyl tiglate; 3-methylbutanoic acid i-pentyl ester; ethyl palmitate; ethyl linoleate; 2,5-dimethoxytoluene; 3-methylfuran; heptane; 2,2,3,3-tetramethylbutane; 1,3-nonadiene; pentadecene; 2-propanone; 2-pentanone; 2-heptanone; 3-octanone; limonene; α-bisabolene and α-cubene (Table 1) (Caileux, *et al.*, 1992, Fiedler, *et al.*, 2001, Jelen & Grabarkiewicz-Szczesna, 2005, Matysik, *et al.*, 2008).

Few literature are available related with *A. niger* metabolism, nevertheless, some fungi reactions are described.

Table 1 - Volatile organic compounds produced by *Aspergillus niger*, analyzed in several studies through different methods.

Compounds	Method	Reference
Alcohols ***Aliphatics***		
Ethanol	DHS/GC-MS	(Caileux, *et al.*, 1992)
1-Propanol	DHS/GC-MS	(Caileux, *et al.*, 1992)
2-Methyl-1-propanol	SPME/GC-MS	(Matysik, *et al.*, 2008)
	DHS/GC-MS	(Caileux, *et al.*, 1992)
2-Methyl-1-butanol	SPME/GC-MS	(Matysik, *et al.*, 2008)
	DHS/GC-MS	(Caileux, *et al.*, 1992)
3-Methyl-1-butanol	SPME/GC-MS	(Jelen & Grabarkiewicz-Szczesna, 2005)
	SPME/GC-MS	(Matysik, *et al.*, 2008)
2-Pentanol	SPME/GC-MS	(Matysik, *et al.*, 2008)
1-Octen-3-ol	SPME/GC-MS	(Matysik, *et al.*, 2008)
	HS-SPME/GC-MS	(Fiedler, *et al.*, 2001)
	SPME/GC-MS	(Jelen & Grabarkiewicz-Szczesna, 2005)
3-Octanol	SPME/GC-MS	(Matysik, *et al.*, 2008)
	HS-SPME /GC-MS	(Fiedler, *et al.*, 2001)
Aldehydes ***Aliphatics***		
Acetaldehyde	DHS/GC-MS	(Caileux, *et al.*, 1992)
Esters ***Aliphatics***		
Ethyl propanoate	DHS/GC-MS	(Caileux, *et al.*, 1992)
Ethyl butanoate	DHS/GC-MS	(Caileux, *et al.*, 1992)
Ethyl tiglate	SPME/GC-MS	(Matysik, *et al.*, 2008)
3-Methyl-2-butenoic acid ethyl ester	SPME/GC-MS	(Matysik, *et al.*, 2008)
Iso-amyl tiglate	SPME/GC-MS	(Matysik, *et al.*, 2008)
3-Methylbutanoic acid i-pentyl ester	SPME/GC-MS	(Matysik, *et al.*, 2008)
Ethyl palmitate	SPME/GC-MS	(Jelen & Grabarkiewicz-Szczesna, 2005)
Ethyl linoleate	SPME/GC-MS	(Jelen & Grabarkiewicz-Szczesna, 2005)
Ethers		
2,5-Dimethoxytoluene	HS-SPME /GC-MS	(Fiedler, *et al.*, 2001)
Furan – type compounds		
3-Methylfuran	SPME/GC-MS	(Matysik, *et al.*, 2008)
Hydrocarbons ***Aliphatics***		
Heptane	SPME/GC-MS	(Jelen & Grabarkiewicz-Szczesna, 2005)
2,2,3,3-Tetramethylbutane	SPME/GC-MS	(Jelen & Grabarkiewicz-Szczesna, 2005)
1,3-Nonadiene	SPME/GC-MS	(Matysik, *et al.*, 2008)
Pentadecene	SPME/GC-MS	(Matysik, *et al.*, 2008)
Ketones ***Aliphatics***		
2-Propanone	HS-SPME /GC-MS	(Fiedler, *et al.*, 2001)
2-Pentanone	SPME/GC-MS	(Matysik, *et al.*, 2008)
2-Heptanone	SPME/GC-MS	(Matysik, *et al.*, 2008)
	HS-SPME /GC-MS	(Fiedler, *et al.*, 2001)
3-Octanone	SPME/GC-MS	(Matysik, *et al.*, 2008)
	HS-SPME /GC-MS	(Fiedler, *et al.*, 2001)
	SPME/GC-MS	(Jelen & Grabarkiewicz-Szczesna, 2005)
Terpenes **Monoterpenes**		
Limonene	SPME/GC-MS	(Jelen & Grabarkiewicz-Szczesna, 2005)
Sesquiterpenes		
α-Bisabolene	SPME/GC-MS	(Jelen & Grabarkiewicz-Szczesna, 2005)
α-Cubebene	SPME/GC-MS	(Jelen & Grabarkiewicz-Szczesna, 2005)

DHS – Dynamic headspace extraction; **GC-MS -** Gas chromatography-mass spectrometry; **HS** – Headspace; **SPME**– Solid phase microextraction.

1.6. Methodologies used for metabolomics studies

The detection of volatile metabolites can be performed by resorting to different methodologies. Several extraction techniques have been used in different researches, namely the technique of dynamic headspace extraction (DHS) and the microextraction techniques, such as solid phase microextraction (SPME) (Caileux, *et al.*, 1992, Fiedler, *et al.*, 2001, Jelen & Grabarkiewicz-Szczesna, 2005, Matysik, *et al.*, 2008). Actually, over the last two decades, microextraction techniques, such as SPME, have been developed to allow a rapid and efficient sample preparation. After extraction, the volatile metabolites can be analysed using chromatographic techniques, including gas chromatography-mass spectrometry (GC-MS) (Caileux, *et al.*, 1992, Fiedler, *et al.*, 2001, Jelen & Grabarkiewicz-Szczesna, 2005, Scotter, *et al.*, 2005, Matysik, *et al.*, 2008). Comprehensive two-dimensional gas chromatography coupled to mass spectrometry with a high resolution time of flight analyser (GC×GC-ToFMS) has been used in several metabolomics studies. This technique has high resolving power and greater sensitivity, when comparing with one-dimensional gas chromatography (1D-GC) (Petronilho, *et al.*, 2014). Given the advantages of the use of SPME combined with GC×GC-ToFMS methodology in microbial metabolomics studies, these two techniques will be discussed in detail.

1.6.1. Solid phase microextraction (SPME)

The solid phase microextraction (SPME) is a sample preparation technique that allows the extraction and concentration of volatile and semi-volatile compounds without the use of solvents (Kataoka, *et al.*, 2000). SPME was developed in the early 1990s by Janusz Pawliszyn (Arthur C. & J., 1990). This technique has covered several areas, like environmental, industrial hygiene, process monitoring and clinical, forensic and food analysis (Pawliszyn, et al., 1997).

The SPME technique involves the use of a fused silica fibre coated with a polymeric stationary phase (Petronilho, *et al.*, 2014). For its protection, this fibre is incorporated in a syringe, constituted by the support of the fibre and the needle (Kataoka, *et al.*, 2000). The support contains the plunger, which allows the exposure and collection of the fibre in the extraction of the compounds and the thermal desorption in the injector of the gas chromatograph. The needle has the function to protect the fibre and to pierce the septum of the vial containing the sample (Pawliszyn, *et al.*, 1997, Castro, *et al.*, 2008).

Beyond the adsorbent and absorbent coatings, also exist the mixed coatings, which contain a liquid polymer and solid particles, matching the absorption properties of the liquid polymer with the adsorption properties of the porous particles (Pillonel, *et al.*, 2002). DVB/CAR/PDMS (divinylbenzene/carboxen/poly(dimethylsiloxane)) coating is a mixed coating with three different polymers, causing a synergistic effect between the absorption and adsorption. This mutual synergistic effect of absorption and adsorption of the stationary phases promotes a high retention ability and consequently, a high sensitivity. Therefore, the DVB/CAR/PDMS coating has been widely used, since it has a wide range of analyte sorption capacities with different physicochemical properties (Petronilho, *et al.*, 2014).

The solid phase microextraction comprises two steps: extraction and desorption (Castro, *et al.*, 2008). In the first step, the sample is placed into a vial, which is sealed with a septum and an aluminium cap. The syringe needle pierces the septum and then the fibre is exposed to the sample by the action of the plunger and the analytes are sorbed by the stationary phase. After the extraction, the fibre is collected into the needle, it is removed from the septum and inserted in the injector of the gas chromatograph, causing the thermal desorption of analytes (Kataoka & Saito, 2011).

In SPME does not occur an exhaustive extraction. The system tends to an equilibrium in which the analytes distribute themselves among the various stages of the system. This technique is based on sorption (absorption and/or adsorption), depending on the stationary phase of the fibre (Arthur C. & J., 1990). The extraction mechanism is based on the interaction between the analyte free fraction and stationary phase of the fibre, i.e., consists in the analytes distribution between the extraction phase and the matrix (Pawliszyn, 2009). In the three-phases system, the partition occurs between the sample, the headspace and stationary phase of the fibre (Figure 3) (Petronilho, 2008).

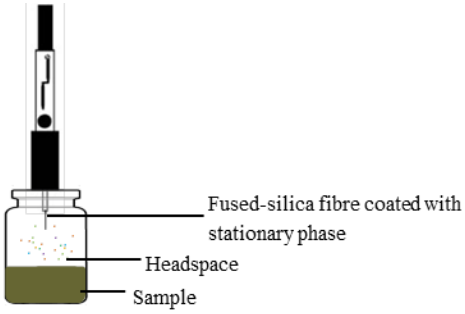

Figure 3 - Partitioning of analytes between stages, three-phases system.

The SPME can be used with three basic extraction modes: direct extraction, headspace extraction (HS-SPME) and extraction involving membrane protection (Pawliszyn, 2009). In the direct extraction mode, the fibre is inserted directly into the sample, and the analytes are carried and extracted directly from the sample matrix to the extraction phase. In the HS-SPME, the fibre is inserted into the headspace above the aqueous matrix. With this mode, only volatile analytes are extracted. In practice, some agitation is applied in order to facilitate and to promote a rapid extraction (Pawliszyn, 2009).

The SPME efficiency and reproducibility are dependent on several parameters, such as SPME fibre coating, stirring effect, salt addition (salting-out effect), pH changes, temperature and extraction time. Stirring promotes the transference of analytes from the sample matrix to the fibre coating, decreasing the extraction time. The addition of salt (e.g. sodium chloride) improves the efficiency of extraction, since there is a decrease in the solubility of the compounds in the sample, increasing its concentration in headspace. The temperature rise causes an improvement of the extraction efficiency, once extraction rate is enhanced. However, it is necessary to find the most suitable temperature to obtain the proper sensitivity (Kataoka, et al., 2000, Petronilho, et al., 2014).

This method is a rapid, simple and sensitive procedure that has important advantages, since it eliminates the use of organic solvents, allows to combine the extraction and the concentration in one single step reducing the analysis time, and can also improve the detection limits (Kataoka, et al., 2000, Hook, et al., 2002, Coelho, et al., 2006).

1.6.2. Comprehensive two-dimensional gas chromatography coupled to mass spectrometry with a high resolution time of flight analyser (GC×GC–ToFMS)

The comprehensive two-dimensional gas chromatography coupled to mass spectrometry with a high resolution time of flight analyser (GC×GC-ToFMS) is becoming accepted as one of the fastest high resolution systems available for the separation of volatile organic compounds (VOC) and semi-volatile compounds (Castillo, *et al.*, 2011). It is a technique with a very high resolving power, which has been explored in metabolomics studies (Rocha, *et al.*, 2012).

GC×GC employs two orthogonal mechanisms to separate the sample constituents based on the application of two GC columns coated with different stationary phases as, for example, a non-polar (separation ruled by boiling points) and a polar (separation by polarity) connected in series through a modulator interface (Petronilho, *et al.*, 2014). These different stationary phases increase the peak capacity as a result of the product from peak capacity of two dimensions through the combination of a non-polar/polar phase (Rocha, *et al.*, 2012). If a cryogenic modulator is used, small portions of the eluate from the first dimension (^{1}D) are cryofocused and re-injected into the second column (^{2}D). Each ^{1}D peak is modulated several times, largely preserving the ^{1}D separation. Compounds co-eluting from ^{1}D undergo additional separation on ^{2}D (Rocha, *et al.*, 2012).

GC×GC may be combined with time-of-flight mass spectrometry (ToFMS), which allows to achieve full mass spectra acquisition at trace level sensitivity and mass spectral continuity (Petronilho, *et al.*, 2014).

Initially, the sample is injected into the injector (Figure 4), and passes through the first column, where separation occurs. Then, interface small (several seconds) portions of the first dimension eluate by cryofocusing, and re-injects them into the second column. While the separation occurs in the second column, a new fraction from the first column suffers cryofocusing in the modulator, and this process repeats itself over again until the run of sample is concluded. The separation in the second column, smaller than the first one, is rapid. This happens because the separation in the second column must be completed before the next fraction to be injected into the modulator, thus the separation in first column is preserved (Silva, *et al.*, 2010, Petronilho, *et al.*, 2014).

The most important property of GC×GC is to give rise to a number of novel capabilities compared with 1D-GC, for example, the structured 2D chromatographic space.

This approach simplifies the data obtained and reduces the time of analysis (Petronilho, *et al.*, 2014). GC×GC offers some advantages over conventional 1D-GC, namely improved resolution, faster run times, increased peak capacity, enhanced mass selectivity and sensitivity, and improved detection limits due to focusing of the peak in the modulator (Petronilho, *et al.*, 2014). Figure 5 shows a high chromatographic resolution due to separation of compounds along the ^{2}D that would co-elute if a 1D-GC was used.

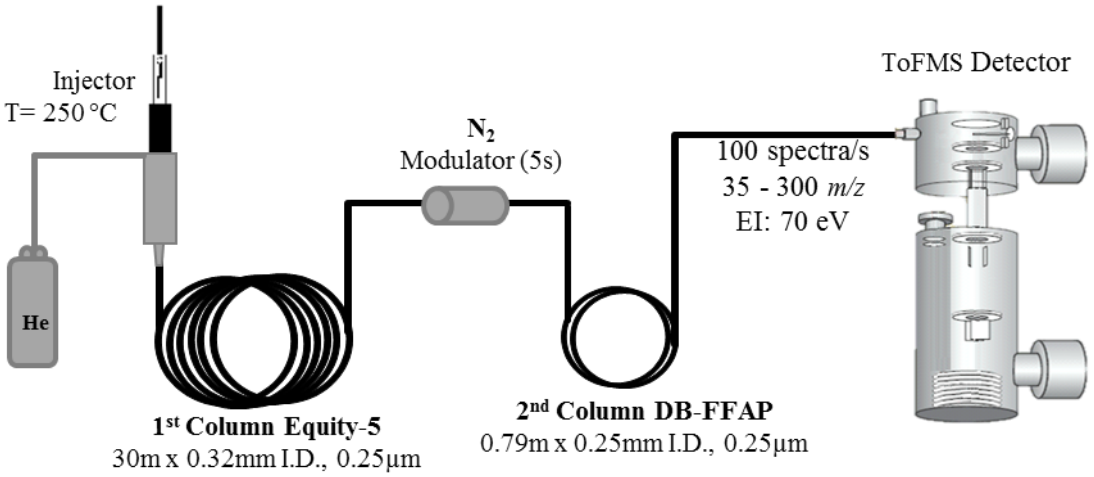

Figure 4- Comprehensive two dimensional gas chromatographic system coupled with time of flight mass spectrometry detection (GC×GC-ToFMS) (Adapted from Petronilho, *et al.* (2014)).

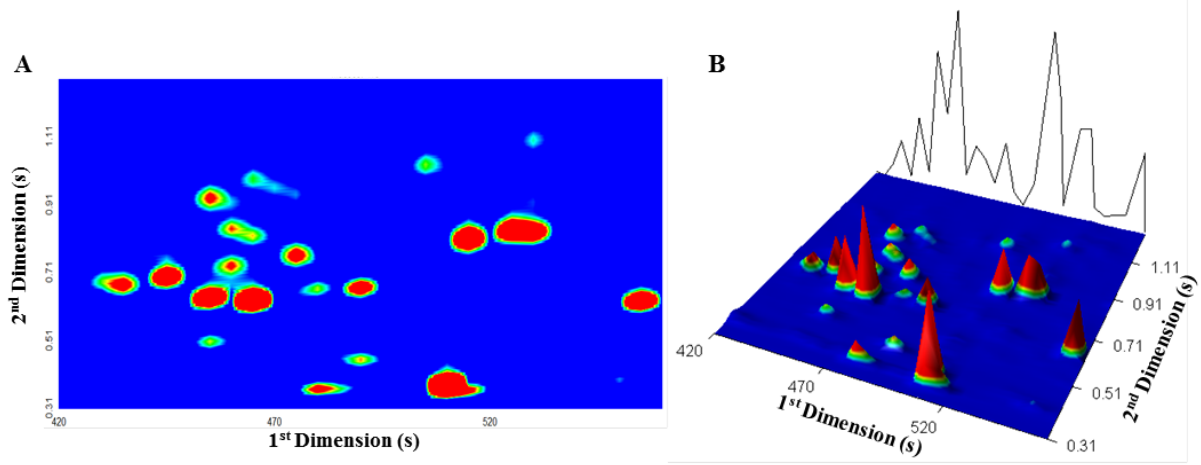

Figure 5 – Example of in two dimensions (A) and three dimensions (B) GC×GC – ToFMS total ion chromatogram plot of *A. niger* sample.

1.7. Data analysis

Considering the complexity of the data obtained using high performance instruments like GC×GC–ToFMS, the appeal to advanced tools of multivariate analysis becomes crucial to extract relevant information. The multivariate analysis helps to identify the main sources of variability in the dataset and variables related to the phenomena of interest as well as perform classification of the samples, based on their metabolic profile (Berrueta, *et al.*, 2007).

Depending on the mathematic model, the statistical techniques used in metabolomics could be divided in unsupervised and supervised techniques. The most commonly used unsupervised technique is Principal Component Analysis (PCA), which allows the study of main sources of variability present in the dataset. This technique is an exploratory tool to find clusters (Härdle W & L., 2007). The central view of PCA is to reduce the dimensionality of a dataset that consists of a large number of correlated variables, while retaining as much as possible of the variation present in the dataset. This is achieved by transforming original variables into a set of new orthogonal ones, the principal components (PCs). The PCs are computed iteratively, in such a way that the first PC carries most information or variance: the second PC carries the maximum share of the residual information, i.e. not taken into account by the first PC and so on. The PCA model is given by the following expression:

$$X = TP' + E,$$

where X is original dataset, T is a matrix of sample scores, P is a matrix of variables loadings and E is error.

One of the most commonly used supervised techniques is the Partial Least Squares (PLS) regression, which is also a projection method similar to PCA. The main difference between PCA and PLS is that in the latter case two matrices X and Y, i.e. dependent and independent variables, are used for data decomposition. The PLS is used to find the fundamental relations between two matrices (X and Y) by calculating a set of new latent variables. The PLS regression model is given by the following expression:

$$X = TP' + E \;\; and \; Y = TQ' + F,$$

where X is original data matrix, T is a sample score matrix, P and Q are loading matrices for variables X and Y, respectively, and E and F are errors. Validation of PLS models is necessary for the determination of the optimal number of latent variables and

evaluation of their predictive performance. It is important to note that as two matrices X and Y are used for data decomposition in PLS resulting scores and loadings are not identical to the one obtained using PCA.

The Discriminant Analysis using the Partial Least Squares (PLS-DA) is a variant of PLS used when the Y is a dummy binary variable coding class membership, which is used to discriminate the classes (Chevallier, et al., 2006) (Wold, *et al.*, 2001, Barker & Rayens, 2003).

Statistical significance of the PLS-DA classification model can be assessed using a permutation test (Westerhuis, *et al.*, 2008, Westerhuis, *et al.*, 2008). Permutation consists in changing randomly the order of the rows in the dataset so the class labels are assigned randomly to the measurements. After that, classification model is calculated using the permuted dataset. Permutation test considers the null hypothesis that a given classification model is not significant and describes noise. If null hypothesis is true, there should be no difference in the value of the quality-of-fit criteria between original dataset and permuted one. After permuting the data and repeating calculations a sufficient number of times H0 distribution of the quality-of-fit criterion is obtained. Value of quality-of-fit criterion for the original dataset should be outside the 95 or 99% confidence bounds of the H0 distribution of the values of the permuted data to be significant.

Selection of the variables that contributed most to the model can be done using Variables Importance in projection (VIP) (Chi-Hyuck, *et al.*, 2009), which allows ranking variables according to their importance in the PLS model. VIP takes into account amount of the variance explained by the variable for each latent variables extracted and covariance between this variable and dependent variable y. VIP of the j^{th} variable, VIP_j, for the model with K latent variables is calculated using the following equation:

$$VIP_j = \sqrt{\frac{p \sum_{k=1}^{K} \left(b_k^2 t_k' t_k \right) (w_{jk})^2}{\sum_{k=1}^{K} \left(b_k^2 t_k' t_k \right)}},$$

where p is a total number of variables in the model, t_k and b_k are scores and regression coefficient for the kth latent variable, respectively, and w_{jk} is a weight for the j^{th} variable and kth latent variable.

1.8. Objectives of this master thesis

Fungal infections have greatly increased in risk populations, namely in immunocompromised patients, because many fungi became resistant to antifungals, as *Aspergillu*s species. Nevertheless, the actual detection methods for fungi are low sensitive and time consuming. Thus, it is required a fast and accurate method to allow a more rapid diagnose and further proper treatment of the infected patients.

Thus, this research aims to perform an in-depth study of *A. niger* exometabolome, in order to establish a targeted metabolomic pattern that can be further exploited to fungal diagnosis. For that, it became important to assess the variation of global metabolomic pattern of *A. niger* in different growth conditions, such as temperature of incubation (25 and 37 °C), type of culture medium (solid and liquid YGC) and incubation time (3 and 5 days).

In addition, according to the conclusions of the first results obtained in this research, it was selected the best growth conditions, and the exometabolome of two other microorganisms, *Penicillium chrysogenum* and *Candida albicans*, was also analysed and compared to the metabolites produced by *A. niger*.

Thus, the principal aim of this research was the exometabolome analysis of *Aspergillus niger* and search for molecular biomarkers pattern.

In order to achieve this goal, more specific objectives were defined, namely:

a) Study of the impact of growth conditions, such as, temperature (25 and 37 °C), growth time (3 and 5 days) and culture medium (solid and liquid) in the metabolism of *A. niger*;
b) Evaluation of the variation of global metabolomic pattern of *A. niger* in different growth conditions and identification of the targeted metabolomic pattern of *A. niger*;
c) Application of the target metabolomic pattern of *A. niger* for distinguish from others microorganisms (*Penicillium chrysogenum* and *Candida albicans*) in defined growth conditions.

Chapter 2: *Aspergillus niger* metabolomics provided by GC×GC–ToFMS: impact of growth conditions

Chapter 2: *Aspergillus niger* metabolomics provided by GC×GC–ToFMS: impact of growth conditions

1. Introduction

Fungal infections have acquired increasing clinical importance due to their greatly increased incidence in risk populations, namely in patients with HIV infection, transplant recipients, cancer patients and other individuals receiving immunosuppressive treatments (Badiee, *et al.*, 2009, Karageorgopoulos, *et al.*, 2011). Many of fungi involved in these infections became resistant to antifungals, such as *Aspergillus* species. *Aspergillus* is the second leading cause of fungal infection in hospital and can cause invasive aspergillosis, being a major cause of morbidity and mortality in immunocompromised patients (Dinand, *et al.*, 2013, Ergene, *et al.*, 2013). Although conventional methods of diagnostic such as microscopy, histology and cellular culture, are essentials for proving the presence or absence of fungal infection, these are time consuming, therefore, their impact on clinical decisions to treat patients is limited (Erjavec & Verweij, 2002).

Microbial metabolomics constitutes an integrated component of biology systems, measures metabolites, to facilitate the understanding of microbial interactions, cellular functions and dynamic of cell metabolism (Tang, 2011, Liebeke & Lalk, 2014). Through the study of the complete set of microorganisms metabolites, microbial metabolomics arises as a powerful feature screening the metabolites produced by microorganisms (Tang, 2011). The metabolome is a set of all metabolites present in cell, which participate in general metabolic reactions and they are essential for the growth, maintenance and normal function of a cell (Dunn & Ellis, 2005). Metabolome is made up of two parts, the endometabolome (intracellular metabolites) and the exometabolome (extracellular metabolites) (Nielsen & Jewett, 2007). Microbial volatile organic compounds (VOC) result from the primary and secondary metabolism of microorganisms and are detectable before any visible sign of microbial growth. For this reason, they can be used as early indicators of microorganisms (Polizzi, *et al.*, 2012). It provides information about the state of biological organisms which can be used as a diagnostic tool for diseases through fungal metabolites pattern.

A methodology based on headspace-solid phase microextraction combined with comprehensive two-dimensional gas chromatography coupled to mass spectrometry with a high resolution time of flight analyser (HS-SPME/GC×GC-ToFMS) represents a suitable powerful tool for study of the exometabolome. It is a technique, with a very high resolving power (Rocha, *et al.*, 2012). GC×GC employs two orthogonal mechanisms to separate the

components of the sample, based on the application of two GC columns coated with different stationary phases. A non-polar (separation by volatility) and a polar (separation by polarity) phases connected in series through a modulator interface (Petronilho, *et al.*, 2014). GC×GC-ToFMS has been successfully used in several fields of analysis, revealing advantages especially for analysis of complex samples (Rocha, *et al.*, 2012, Rocha, *et al.*, 2013).

This research study aims to establish the *A. niger* exometabolome and to study of the impact of growth conditions, such as, temperature, growth time and culture medium in the metabolism of *A. niger*. After that, search for *A. niger* target metabolomic pattern was performed. To fulfil these objectives, the fungus was cultivated in different growth conditions, namely temperature (25 and 37 °C), growth time (3 and 5 days) and type of medium (solid and liquid). Then the samples were analysed by using HS-SPME/GC×GC-ToFMS.

2. Material and methods

2.1. Fungal strain

For this study, a strain of *Aspergillus niger* was used. This fungus was provided by the Applied and Environmental Laboratories of the Biology Department of Aveiro University. The initial culture was inoculated in Yeast Glucose Chloramphenicol Agar (YGC, Liofilchem®) and incubated at 25 °C, for 7 days, in order to obtain isolated colonies for later use in the preparation of new cultures in solid and liquid media.

2.2. Culture conditions and preparation of samples

The experimental assays were carried out under different conditions. The *A. niger* was incubated in different growth conditions: culture medium (solid and liquid YGC), temperature (25 and 37 °C) and incubation time (3 and 5 days). Three independent assays were performed for each condition.

For the samples incubated in solid medium, were added of 10 mL of Ringer solution (Merck Millipore) per plate (5 plates). Then a loopful was used to help to remove the cellular content. Twenty-five millilitres of each sample were collected for exometabolome analysis and twenty-five millilitres for the determination of cell concentration.

For the samples incubated in liquid YGC medium (10 g D-glucose and 5 g yeast extract for 1 L), twenty-five millilitres of each sample were collected for exometabolome analysis and twenty-five millilitres for the determination of cell concentration.

2.3. Determination of cell concentration

The determination of cell concentration was obtained through the colony forming-units (CFU) counts. The samples were homogenized by means of mashing. Culture aliquots were serially diluted in Ringer solution and 100 µL of each diluted sample were plated on YGC, (five replicates per dilution). The plates were incubated at 25 °C for 7 days and the colonies were counted in the replicates of the most suitable dilution. The concentration of viable cells was expressed as colony forming-units per millilitre (CFU mL^{-1}). These results were used for the normalization of total areas of compounds obtained from the exometabolome analysis.

2.4. Determination of exometabolome

2.4.1. Samples preparation

After incubation, for each sample 25 mL of culture were collected and centrifuged at 10 000 rpm, at 4 °C, for 15 min (Centrifuge Beckman AVANTI). For headspace solid-phase microextraction (HS-SPME) assay 20 mL (1/β ratio of 0.5) of supernatant were transferred into a 60 mL glass vial, through syringe and syringe filter with a pore 0.20 µm. After the addition of 4 g of NaCl (≥99.5%; Sigma-Aldrich) and stirring bar of 20×5 mm, the vial was capped with a polytetrafluoroethylene septum and an aluminium cap (Chromacol Ltd., Herts, UK). The samples were stored at -80 °C for further analysis. The same procedure was applied to YGC media samples.

2.4.2. HS-SPME procedure

The SPME holder for manual sampling and the coating fibre were purchased from Supelco (Aldrich, Bellefonte, PA, USA). SPME device included a fused silica fibre coating partially cross-linked with 30/50 µm DVB/CAR/PDMS. The selection of this fibre coating was based on a previous metabolomic studies (Salvador, *et al.*, 2013). This fibre presents a wide range capacity of sorbing compounds with different physicochemical properties.

After defrost the sample vials were placed in a thermostated water bath at 50 °C, under agitation at 350 rpm. The DVB/CAR/PDMS fibre was inserted in the headspace for 30 min for metabolites extraction. Three independent aliquots were analysed for each conditions under study.

2.4.3. GC×GC-ToFMS analysis

The SPME coating fibre was manually introduced into the GC×GC–ToFMS injection port and maintained during 30 s at 250 °C for the desorption occurs. The injection port was lined with a 0.75 mm I.D. splitless glass liner and splitless injections were used (30 s). The LECO Pegasus 4D (LECO, St. Joseph, MI, USA) GC×GC-ToFMS system consisted of an Agilent GC 7890A gas chromatograph (Agilent Technologies, Inc., Wilmington, DE), with a dual stage jet cryogenic modulator (licensed from Zoex) and a secondary oven, and mass spectrometer equipped with a high resolution ToF analyser. In the first dimension was used an Equity-5 column (30 m × 0.32 mm I.D., 0.25 µm film thickness, Supelco, Inc., Bellefonte, PA, USA). In the second dimension was used an DB-

FFAP column (0.79 m x 0.25 mm I.D., 0.25 μm film thickness, J&W Scientific Inc., Folsom, CA, USA). The carrier gas was helium at a constant flow rate of 2.50 mL min^{-1}. The primary oven temperature was programmed from 40 °C (1 minute) to 140 °C at 10 °C min^{-1}, followed by 140 °C to 200 ºC (1 minute) at 7 °C min^{-1}. The secondary oven temperature was programmed from 55 °C (1 minute) to 155 °C at 10 °C min^{-1}, then 155 °C to 215 °C (2 minutes) at 7 °C min^{-1}. The MS transfer line temperature was 250 °C and the MS source temperature was 250 °C. The modulation time was 5 seconds; the modulator temperature was kept at 20 °C offset (above primary oven) and the hot and cold pulse duration time was 0.80 and 1.70 seconds, respectively. The ToFMS was operated at a spectrum storage rate of 100 spectra s^{-1} and the mass spectrometer was operated in the EI mode at 70 eV using a range of *m/z* 35-300 and the detector voltage was -1480 V. Contour plots were used to evaluate the general separation quality and for manual peak identification. To identify the compounds, the mass spectrum of each one is compared with existing ones in the library (Wiley 275 and National Institute of Science and Technology (NIST) v. 2.0-Mainlib and Replib). Furthermore, a manual analysis of the mass spectra was done, combined with additional information, such as the retention index (RI) value, which was determined according to van den Dool and Kratz RI equation (van Den Dool & Dec. Kratz, 1963). For the determination of the RI, a C_8-C_{20} *n*-alkanes series was used, and these values were compared with values reported in the literature for chromatographic columns similar to that used as the ^{1}D column in the present work. The areas obtained were used to estimate the relative amount of each volatile compound. Total ion chromatograms (TIC) were processed by using the automated data processing software ChromaTOF (LECO) at (S/N) signal-to-noise threshold 200. Chromatograms were used to evaluate the general separation quality and for manual peak identification. The identification of the different compounds was made by analysing the mass spectrum of each compound detected and was compared to those in mass spectral libraries, which are included in the library of standard of GC×GC–ToFMS (Wiley 275 and NIST – Mainlib and Replib).

2.4.4. Data processing

In order to assess the effect of the tested growth conditions (2 temperatures × 2 growth times × type of media, each one with 3 independent replicates) on the production of volatile metabolites, the total number of volatile metabolites and respective sum of GC peak area area were used. Information was extracted from the chromatograms and used to build the data matrices (peak areas without normalization/number of compounds × all tested growth conditions, peak areas with normalization × all tested growth conditions, peak areas of 44 metabolites × all tested growth conditions). One-way analysis of variance (ANOVA) with multiple comparisons test (Tukey post-hoc) was used to assess the possible significant differences between tested conditions. Statistical significant differences were considered for $p < 0.05$. Normality was checked by the Shapiro-Wilk test and homogeneity of variances by the Levene test. When the homogeneity of variances was not verified, the Games-Howell post-hoc test was applied. All statistical analysis was conducted with the IBM SPSS Statistics 20.

A heatmap visualization of the dataset, (3 tested growth conditions under study and 504 volatile compounds), normalized by maximum of each metabolite for all samples (with the GC peak already normalized by CFU) was also performed by using the Unscrambler® X (30 day trial version - CAMO Software AS, Oslo, Norway).

To determinate the metabolomic pattern of *A. niger*, the initial compounds matrix, obtained by HS-SPME/GC×GC-ToFMS, was reduced to a subset of 430 metabolites that was considered as *A. niger* exometabolome. The data matrix for multivariate analysis consisted of 24 observations from *A. niger* (2 temperatures × 2 times × type of media, each one with 3 independent replicates) and 430 variables (GC peak areas from the compounds from several chemical families: acids, alcohols, aldehydes, esters, ethers, hydrocarbons, ketones, N-compounds, S-compounds, terpenic compounds and C_{13} norisoprenoid, in table S3). Also, target metabolomic pattern of *A. niger* was achieved with 44 metabolites, by considering the metabolites that appear in all tested growth conditions. In this approach, an identical data matrix was used but taking into account 44 variables (GC peak areas from the compounds in table 2). The metabolites chromatographic areas were normalized by total area and autoscaling. PCA analysis was performed in the software MetaboAnalyst 2.0 (online version).

3. Results and discussion

3.1. Evaluation of the impact of growth conditions on the global volatile composition

In order to obtain information about potential exometabolome, before the analysis by GC×GC-ToFMS *A.niger* was incubated with different growth conditions: temperature (25 and 37 °C), incubation time (3 and 5 days) and culture medium (solid and liquid YGC). Figure 6 shows the number of detected volatile compounds in all growth conditions and no statistical differences ($p > 0.05$) were observed between them. It was detected around 600 compounds, in average, per sample.

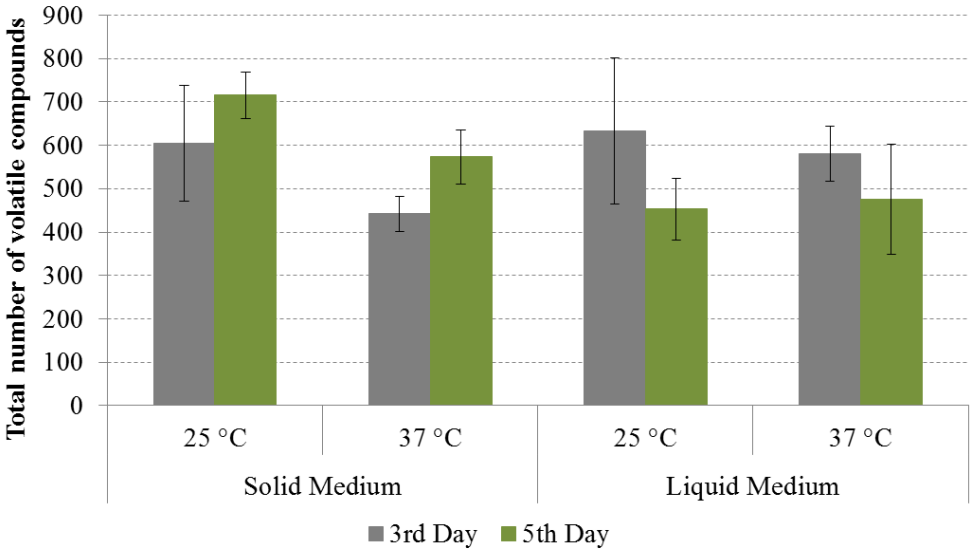

Figure 6 – Total number of volatile compounds detected, during 3 and 5 days of growth, using solid and liquid YGC medium, at 25 and 37 °C. Each column represents the average calculated for three replicates and error bars represent the standard deviation. No significant differences ($p > 0.05$) were observed among all the conditions under study.

Total chromatographic area of volatile compounds produced by *A. niger* is represented in Figure 7-A, and Figure 7-B total chromatographic area was normalized by CFU mL^{-1}. The results showed significant differences between the conditions under analysis. For example, normalized total chromatographic area (Figure 7-B), for 3 and 5 days of growth, at 25 °C, using solid YGC medium had significant differences ($p < 0.05$) comparatively to the other conditions. Analysing the three replicates (Figure 7-B), the highest peak area of volatile compounds was observed in 5^{th} day of growth in solid medium, at 25 °C. At 25 and 37 °C, for 3 days of growth, using liquid medium, it is possible to detect higher GC peak area comparing with 5 days of growth in the same

conditions. The same trend was registered at 37 ºC, for 3 days of growth, using solid medium, significant differences ($p < 0.05$) were found.

Nutrients of the culture medium were important for the growth. Korpi, *et al.* (2009), suggested that microbial species, growth phase, nutrients, pH, humidity and temperature have a great impact on the metabolites production. The lack of nutrients for later time (5 days) may affect the metabolism and consequently, the chromatographic area was lower comparing with samples with 3 days of growth. Overall, normalized GC peak total area was higher for 3 and 5 days of growth, at 25 °C for solid YGC medium.

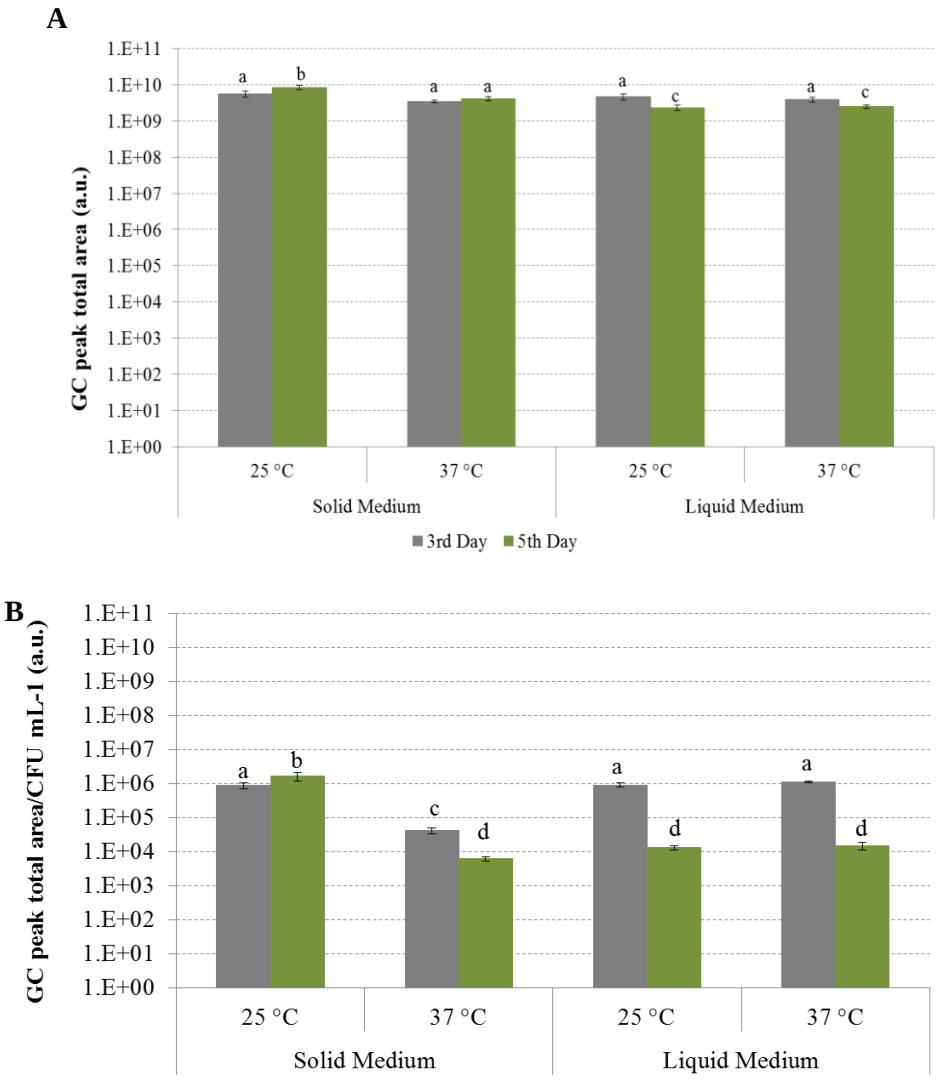

Figure 7 - Total area (**A**) and total area normalized by CFU mL^{-1} (**B**) of detected volatile compounds, during 3 and 5 days of growth, using solid and liquid YGC medium, at 25 and 37 °C. Each column represents the average calculated for three replicates and error bars represent the standard deviation. Different letters (a-d) represent significant differences ($p < 0.05$) among conditions.

A. niger is a strictly aerobic microorganism, and its growth in liquid medium occurs at the surface, where oxygen is available. Thus, the available oxygen at 5 days of growth is lower, and less GC peak area was detected (Figure 7-B), when comparing with GC peak area observed for 3 days of growth, within this type of medium. In terms of solid medium, the same trend was only registered for 37 ºC.

3.2. *Aspergillus niger* exometabolome

The obtained GC×GC total ion chromatogram contour plots that are illustrated on (Figure 8 and 9) show the total ion chromatogram contour plots obtained from growth of *A. niger* in different conditions. The several conditions under study were in fact complex, due to the detection of several hundred of peaks, as mentioned before (section 3.1).

The most reliable way to validate the identification of an analyte is the co-injection of an authentic standard, however, as a metabolomic approach was the objective of this master thesis, it would be unachievable in the time available for analysis, and also most of the cases the standards are not commercially available, or is economically prohibitive (Rocha, *et al.*, 2013).

Thus, in this study, several parameters were used as a strategy was to perform a putative identification: MS spectral similarity (similarity value > 700/1000) and the RI parameter calculated, since the calculated retention index (RIcalc) differed 0-6% when compared to literature data (RIlit) for the ^{1}D column or equivalent (Table S2). Also, GC×GC structured chromatogram principle, an unique peculiarity of GC×GC, can be a powerful tool in the identification step. For instance, it was possible to identify different chemical groups with differences in polarity (Figure 8 and 9), for example, as aliphatic hydrocarbons had the lower polarity, consequently, they had the lower retention time for the ^{2}D; while higher polarity was observed for alcohols, aldehydes and ketones, which had higher retention time for the ^{2}D. This feature can also be observed in Table S2.Thus, with this strategy, 504 compounds were putative identified, as their corresponding chromatographic data is available on Table S2.

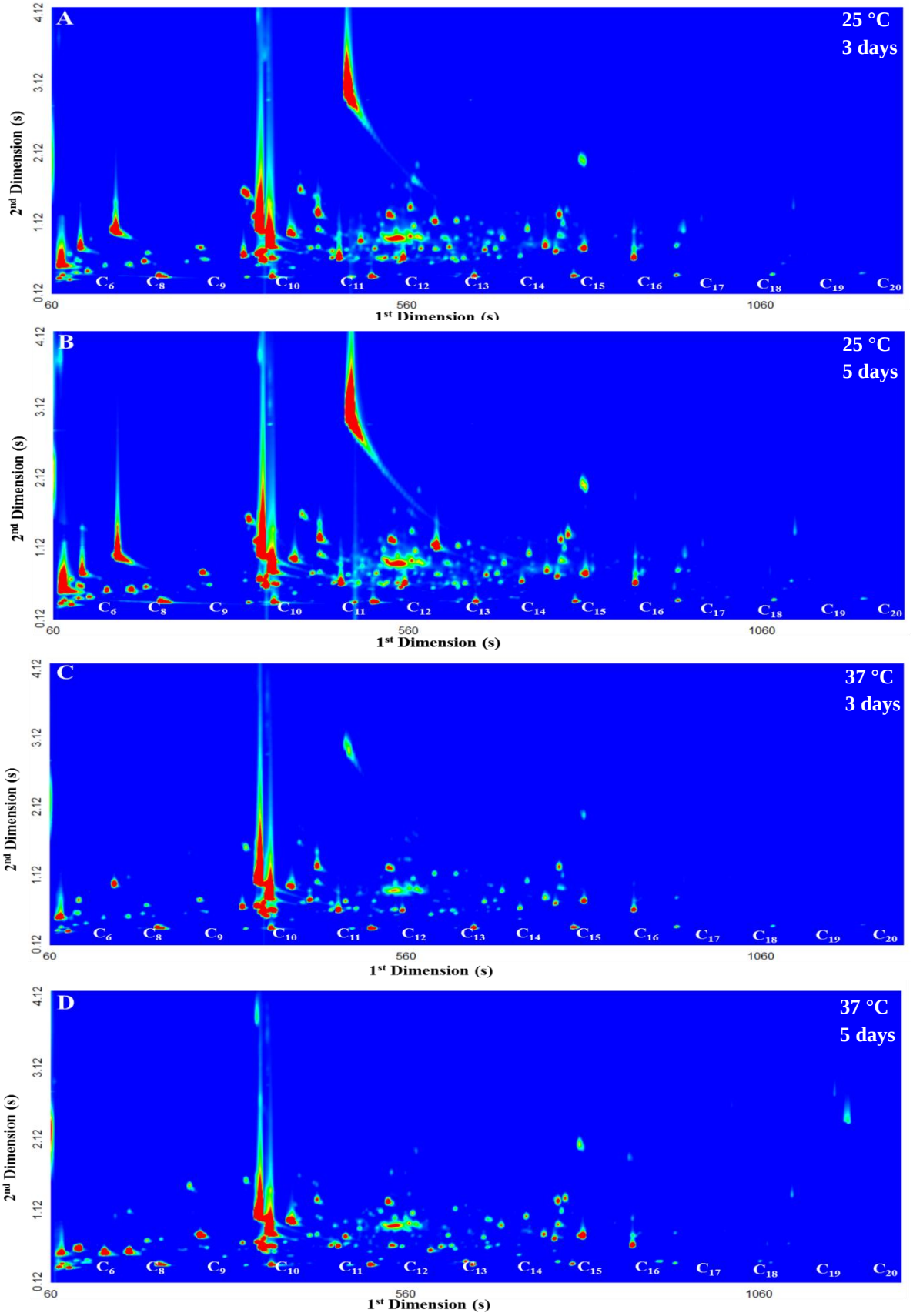

Figure 8 -Typical GC×GC-ToFMS total ion chromatogram contour plots. **A** – 3 days of growth, at 25 °C, using solid medium; **B** – 5 days of growth, at 25 °C, using solid medium; **C** – 3 days of growth, at 37 °C, using solid medium; **D** – 5 days of growth, at 37 °C, using solid medium; Part of the *n*-alkanes series (C_6-C_{20}) was superimposed on the contour plots.

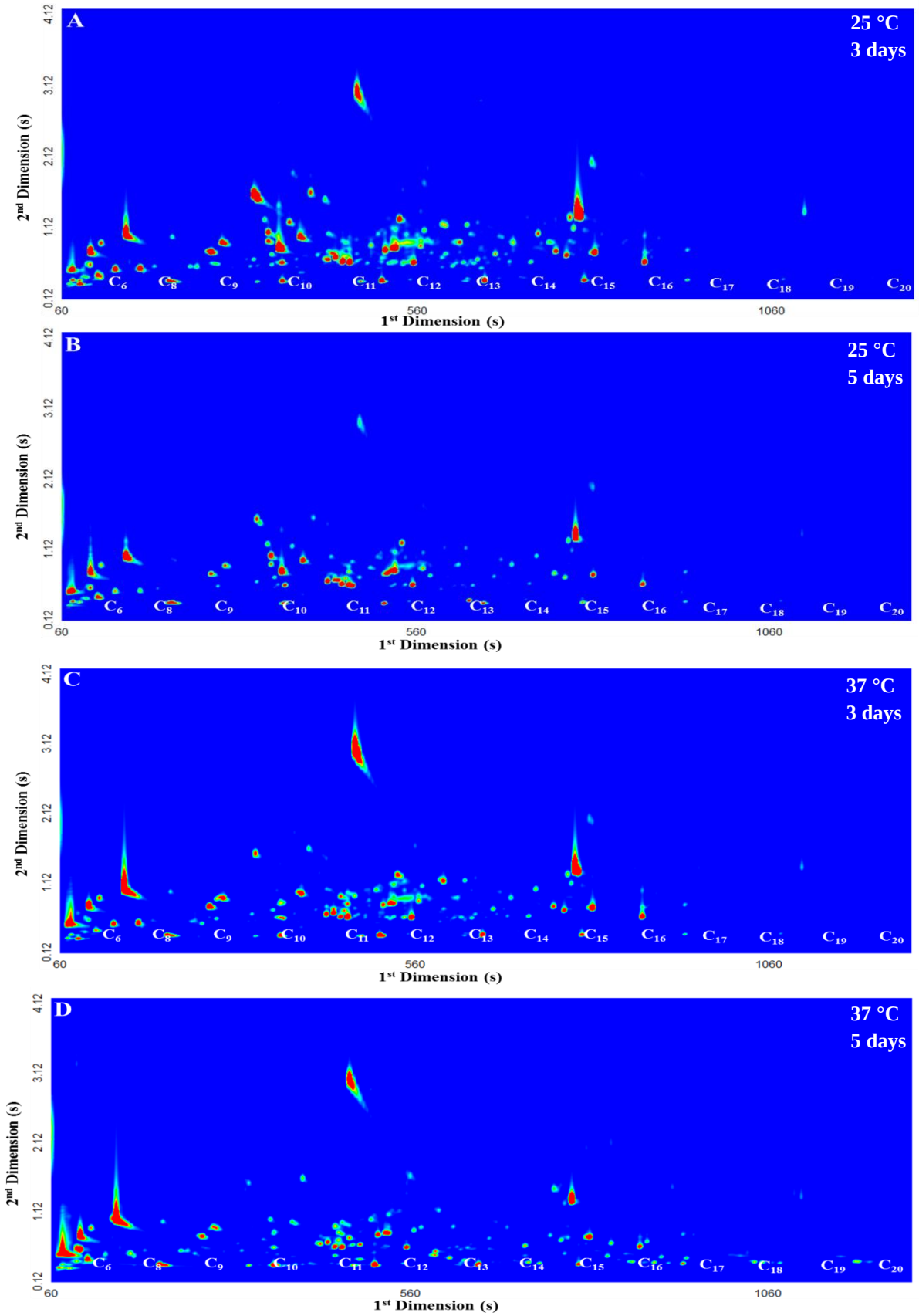

Figure 9 – Typical GC×GC-ToFMS total ion chromatogram contour plots. **A –** 3 days of growth, at 25 °C, using liquid medium; **B –** 5 days of growth, at 25 °C, using liquid medium; **C –** 3 days of growth, at 37 ^{0}C, using liquid medium; **D –** 5 days of growth, at 37 °C, using liquid medium. Part of the *n*-alkanes series (C_6-C_{20}) was superimposed on the contour plots.

The visual analysis of contour plots was very useful for a rapid assessment of *A. niger* exometabolome and for the comparison of the different growth conditions. The obtained GC×GC total ion chromatogram contour plots, illustrated in Figure 8 and 9, relative to *A. niger* samples grown in solid medium and liquid, respectively, is a snapshot of the volatile profile of *A. niger*. The visual analysis of contour plots allowed perceiving resemblances between the samples from the same type of medium, either for solid (Figure 8) and liquid medium (Figure 9); and also differences can be observed depending on medium type (Figure 8 and 9).

Differences in total chromatographic area were observed, for instance, (Figure 8- A and B) higher intensity of chromatographic area was registered for samples at 25 °C when compared ate 37 °C using solid YGC medium (Figure 8-C and D). In terms of the samples that grown in liquid YGC medium (Figure 9), the higher intensity of the chromatographic area was observed at 3 days of growth, at 25 °C, comparing with other samples for this medium. In overall, the intensity of the chromatographic area for samples that grown in liquid YGC medium, was lower than samples from solid YGC medium.

A total of 504 metabolites were identified, being 15.48% alcohols, 14.68% hydrocarbons, 13.89% esters, 13.09% ketones, 8.73% aldehydes, 5.56% C_{10} monoterpenic compounds, 5.56% pyrazines, 4.56% halogenated compounds, 3.77% furan-type compounds, 3.57% S-compounds, 2.78% C_{15} sesquiterpenes, 2.58% N-compounds, 2.38% ethers, 1.98% acids, 0.79% furanones and 0.59% C_{13} norisoprenoids. The supplementary data (Table S2) includes a list of the total metabolites, and the corresponding retention times in both dimensions, the RI obtained through the modulated chromatogram and the RI reported in the literature for a comprehensive GC×GC system with Equity-5 for the first dimension column.

In order to analyse the dataset concerning the exometabolome of *A. niger*, in an easier way, a heatmap representation was performed. The heatmap (Figure 10) showed a graphical representation of the data from Table S3, where GC peak areas (already normalized by CFU) were normalized by maximum, each compound was illustrated through different colours intensities (from blue to red). It allowed a rapid visual access of the relative abundance of each chemical family and also to the comparison of the similarities and differences between samples under analysis. Heatmap showed that *A. niger* under study exhibit differences in the metabolomic profile. Variances among volatile

metabolites produced under different growth conditions were observed. For example, the major relative abundance was observed for 5 days of growth, at 25 °C, using solid YGC medium. It was possible to observe a major relative abundance for terpenic compounds in this condition of growth and it should be expected that these samples will present higher terpenic compounds load, as it could be seen on Table S2. These results indicated the high complexity of *A. niger* exometabolome. In addition, it is possible obtain the relative proportions of each chemical family from the tentatively identified compounds on different samples. While terpenic compounds areas were larger for solid medium, at 25 °C for 5 days of growth, alcohols, aldehydes, hydrocarbons and ketones areas were more intense for solid medium, at 25 °C for 3 and 5 days of growth, and for liquid medium, for 3 days, at 25 °C and 37 °C. Distinct chemical families areas proportions according different growth conditions of *A. niger* might be explained by different constraints affecting the metabolic pathways, namely temperature and growth time.

In literature are reported some metabolites produced by *A. niger*, such as, ethanol; 1-propanol; 2-methyl-1-propanol; 2-methyl-1-butanol; 3-methyl-1-butanol; 2-pentanol; 1-octen-3-ol; 3-octanol; acetaldehyde; ethyl propanoate; ethyl butanoate; ethyl tiglate; 3-methyl-2-butenoic acid ethyl ester; iso-amyl tiglate; 3-methylbutanoic acid i-pentyl ester; ethyl palmitate; ethyl linoleate; 2,5-dimethoxytoluene; 3-methylfuran; heptane; 2,2,3,3-tetramethylbutane; 1,3-nonadiene; pentadecene; 2-propanone; 2-pentanone; 2-heptanone; 3-octanone; limonene; α-bisabolene and α-cubene (Caileux, *et al.*, 1992, Fiedler, *et al.*, 2001, Jelen & Grabarkiewicz-Szczesna, 2005, Matysik, *et al.*, 2008).

In the present study, it was possible to detect 1-propanol; 2-methyl-1-propanol; 3-methyl-1-butanol; 2-pentanol; 1-octen-3-ol; 3-octanol; acetaldehyde; ethyl butanoate; ethyl tiglate; heptane; pentadecene; 2-propanone; 2-pentanone; 2-heptanone and 3-octanone. These compounds were detected for shorter growth times when compared with other studies where which used longer growth times (7 and 10 days) (Caileux, *et al.*, 1992, Jelen & Grabarkiewicz-Szczesna, 2005). Also, in present study YGC medium was used and in studies mentioned above, the different media, such as Sabouraud dextrose agar, were used.

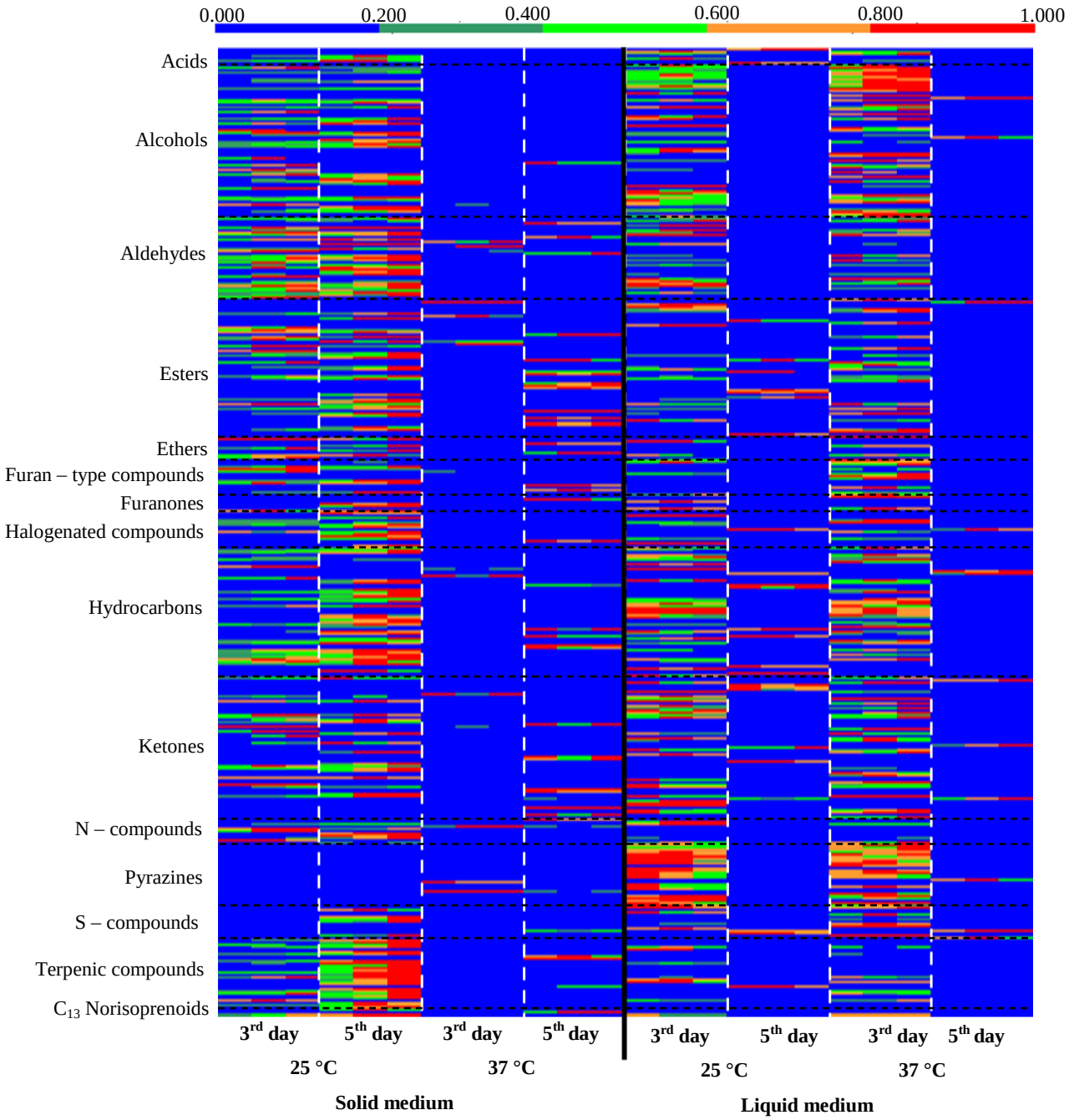

Figure 10 - Heatmap representation of volatile compounds identified from *A. niger* cultures in YGC medium (around 504 metabolites) in different growth conditions, such as culture medium (solid and liquid YGC), temperature of 25 and 37 ºC and incubation time of 3 and 5 days. Different intensities correspond to the normalized GC peak of each compound.

In order to identify the sources of variability related with growth conditions, and to try to eliminate the influence of the growth medium, some chemical families (furan-type compounds, furanones, halogenated compounds and pyrazines) were removed from the original dataset. This selection was based on previous studies about YGC volatile

composition (Ames & Elmore, 1992), and also because one analysis done on the solid and liquid YGC media allowed to conclude that these compounds are present in higher levels in the growth medium compared to the medium with *A. niger* (data not shown). Thus, a new dataset comprises 430 metabolites was obtained (Table S3), which was submitted to PCA processing.

PCA scores (Figure 11) show a separation between the tested conditions, revealing that PC1 and PC2 explain *ca.* 40.3 % of the variability of the dataset. The samples are separated along PC1 depending on the type of medium that is the main source of variability being solid medium located on PC1 negative and liquid medium on PC1 positive. In conclusion, the culture medium has impact in *A. niger* metabolism. Figure 12 represents the corresponding loadings plot profile which established the contribution of each chemical family for the observed sample distribution of Figure 11.

In terms of temperature and time of growth, they have a smaller effect. For the liquid medium samples, it is possible to observe a separation between the different growth times along PC1, while for the solid medium samples, they were separated according to the both growth temperature and time along PC1.

Differences in volatile composition between *A. niger* growth in different conditions a related to several chemical families of metabolites. The acids, alcohols, ethers, hydrocarbons and ketones was contributed from the liquid medium samples, while content of aldehydes and esters were contributed from the samples that were grown in solid medium.

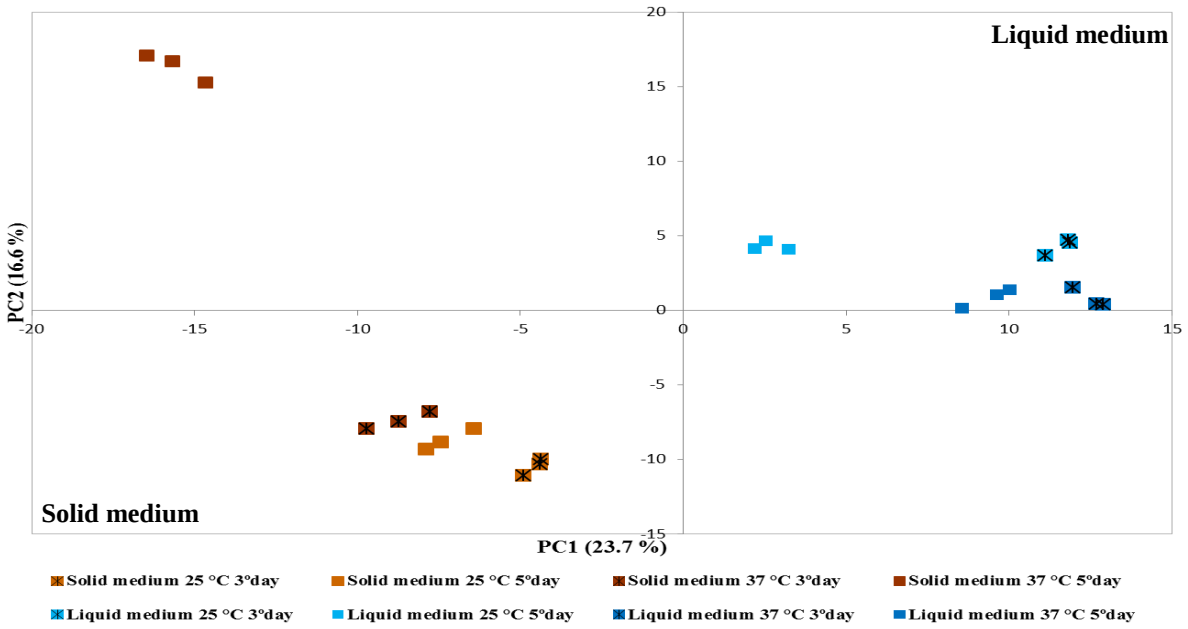

Figure 11 - PCA scores plot based on GC×GC peak areas of a set of 430 compounds identified for *A. niger* exometabolome in all growth conditions.

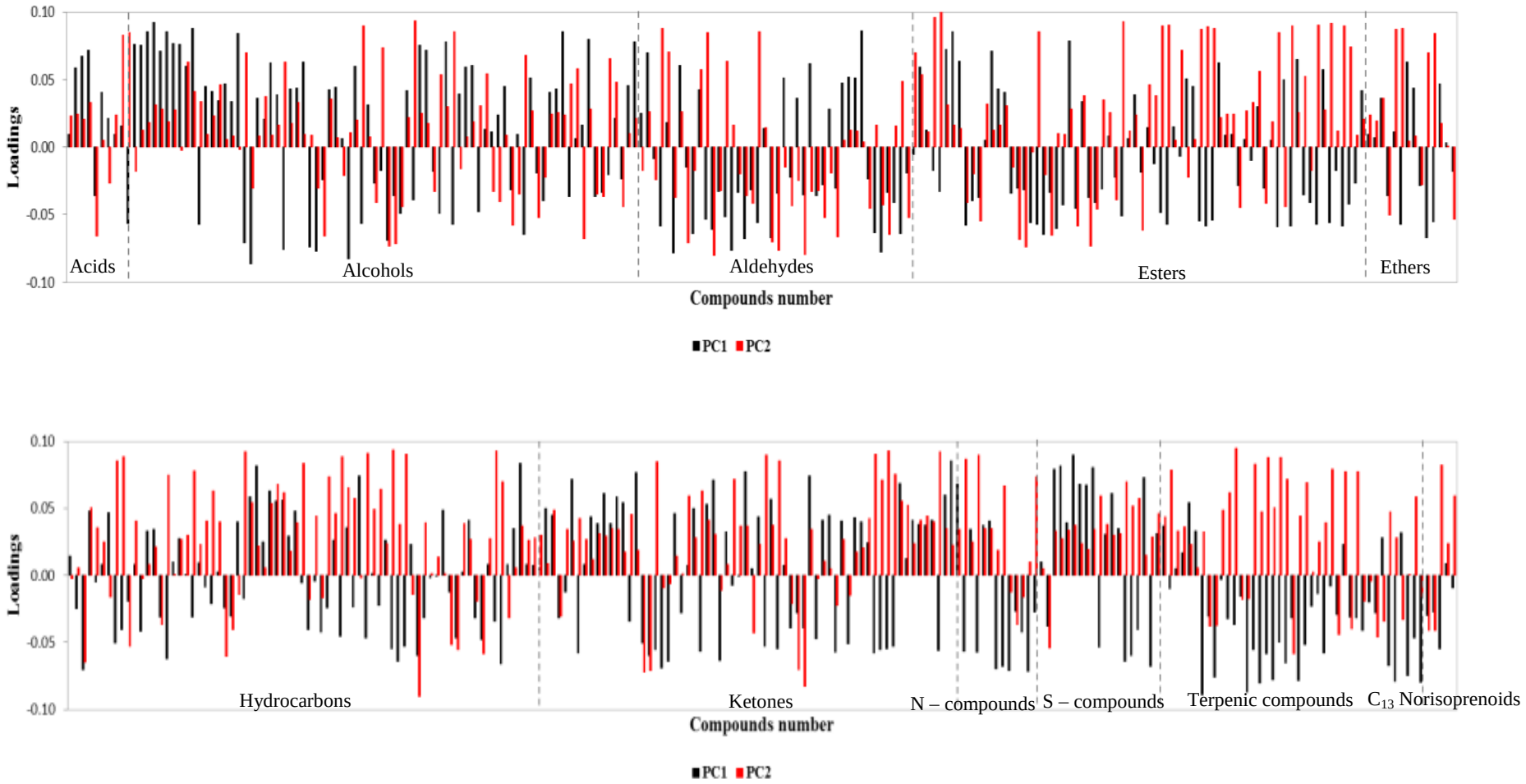

Figure 12 – PC1 and PC2 loadings plot explaining the separation observed in scores map. The variables are organized according the chemical families.

3.3. Searching for *Aspergillus niger* targeted metabolomic pattern

With the aim to determine targeted metabolomic pattern of *A.niger*, metabolites present in the culture independently of growth conditions were identified. Thus a subset of 44 metabolites were found, which were distributed over several chemical families, such as alcohols, aldehydes, esters, hydrocarbons, ketones, terpenic compounds and C_{13} norisoprenoids (Table 2).

Table 2 - Set of metabolites for the *A. niger* targeted metabolomic pattern.

^1t_R[a] (s)	^2t_R[a] (s)	Compound	R.I. Calc[b]	R.I. Lit[c] GC×GC	R.I. Lit[c] GC-MS	R.I. Reference
		Alcohols				
		Aliphatics				
115	0.910	1-Butanol	644	655	-	(Xu, *et al.*, 2003)
150	1.160	3-Methyl-1-butanol	718	706	-	(Rocha, *et al.*, 2013)
255	1.140	1-Hexanol	878	877	-	(Rocha, *et al.*, 2013)
345	1.100	1-Heptanol	975	974	-	(Salvador, *et al.*, 2013)
350	1.050	1-Octen-3-ol	980	992	-	(Silva, *et al.*, 2010)
365	1.270	3-Octanol	996	-	996	(Zhao, *et al.*, 2006)
395	0.990	2-Ethyl- 1-hexanol	1029	1038	-	(Silva, *et al.*, 2010)
435	0.790	2,6-Dimethyl-7-octen-2-ol	1073	-	1075	(Diaz & Kite, 2002)
440	1.030	1-Octanol	1079	1079	-	(Silva, *et al.*, 2010)
		Aromatics				
475	3.030	2-Phenylethanol	1120	1107	-	(Weldegergis, *et al.*, 2011)
805	2.060	2,4-bis(1,1-dimethylethyl)-phenol	1514	-	1513	(Zhao, *et al.*, 2006)
		Aldehydes				
		Aliphatics				
110	0.460	3-Methylbutanal	633	628	-	(Loureiro, *et al.*, 2014)
190	0.590	Hexanal	801	800	-	(Rocha, *et al.*, 2012)
275	0.620	Heptanal	901	903	-	(Rocha, *et al.*, 2012)
465	0.630	Nonanal	1106	1106	-	(Rocha, *et al.*, 2013)
555	0.630	Decanal	1207	1206	-	(Rocha, *et al.*, 2012)
685	0.770	2-Undecenal	1364	-	1376	(Ramarathnam, *et al.*, 1993)
720	0.650	Dodecanal	1407	1406	-	(Rocha, *et al.*, 2012)
		Aromatics				
335	1.550	Benzaldehyde	965	964	-	(Caldeira, *et al.*, 2011)
410	1.620	Benzeneacetaldehyde	1046	1049	-	(Xu, *et al.*, 2003)
		Esters				
		Aliphatics				
135	0.530	Methyl 2-methylpropenoate	685	710	-	(Xu, *et al.*, 2003)
695	0.920	3-hydroxy-2,4,4-trimethylpentyl 2-methyl-propanoate	1376	-	1381	(Kallio, *et al.*, 2006)
		Aromatics				
545	1.050	2-Phenylethyl acetate	1196	-	1192	(Adams, 1995)
		Hydrocarbons				
		Aliphatics				
880	0.490	Hexadecane	1601	1600	-	(Rocha, *et al.*, 2012)
965	0.430	Heptadecane	1701	1700	-	(Rocha, *et al.*, 2012)
		Aromatics				
115	0.460	Benzene	643	-	648	(Isidorov, *et al.*, 2003)
170	0.540	Toluene	759	771	-	(Xu, *et al.*, 2003)
250	0.590	1,3-Dimethylbenzene	871	-	874	(Engel & Ratel, 2007)
270	0.640	1,2-Dimethylbenzene	901	900	-	(Rocha, *et al.*, 2013)
325	0.580	Propylbenzene	953	959	-	(Xu, *et al.*, 2003)
335	0.590	1-Ethyl-4-methylbenzene	964	970	-	(Xu, *et al.*, 2003)
365	0.640	1,3,5-Trimethylbenzene	995	974	-	(Xu, *et al.*, 2003)
390	0.580	1-Methyl-2-(1-methylethyl)benzene	1023	-	1022	(Adams, 2000)
390	0.690	1,2,3-Trimethylbenzene	1023	-	1022	(Adams, 2000)
425	0.610	2-Ethyl-1,4-dimethylbenzene	1062	-	1087	(Wang, *et al.*, 1994)
700	1.270	Biphenyl	1383	-	1385	(Leffingwell & Alford, 2005)
880	1.020	2-Methyl-6-phenyl-1,6-heptadiene	1601	-	-	-

^1t_R [a] (s)	^2t_R [a] (s)	Compound	R.I. Calc [b]	R.I. Lit [c] GC×GC	R.I. Lit [c] GC-MS	R.I. Reference
		Ketones				
		Aliphatics				
75	0.390	2-Propanone	559	-	503	(Rembold, *et al.*, 1989)
265	0.580	3-Heptanone	889	884	-	(Xu, *et al.*, 2003)
355	0.740	6-Methyl-5-hepten-2-one	985	985	-	(Xu, *et al.*, 2003)
495	0.760	3-Nonen-2-one	1140	-	1144	(Elmore, *et al.*, 2002)
755	0.800	6,10-Dimethyl-,5,9-undecadien-2-one	1451	-	1455	(Adams, *et al.*, 2005)
		C_{10} Monoterpenenic compounds				
625	0.630	Endobornyl acetate	1289	-	1285	(Adams, 1995)
		C_{13} Norisoprenoid				
780	0.750	α-iso-methyl ionone	1482	-	-	-

[a] Retention times for first (^1t_R) and second (^2t_R) dimensions in seconds.
[b] RI: Retention Index obtained through the modulated chromatogram.
[c] RI: Retention Index reported in the literature for Equity-5 column or equivalents.

The GC peak area, normalized by CFU of a subset of 44 metabolites identified for *A. niger* in the 8 growth conditions was represented in Figure 13. The solid medium at 37 °C, to 3 days of growth was significant differences between other conditions ($p < 0.05$). Thus, it could be concluded that temperature, culture medium and time of growth have significant influence on production of these compounds, for solid medium.

In liquid medium, with 3 days of growth, at 25 and 37 °C, no significant differences ($p > 0.05$) were found between these two temperatures. Thus, it could be concluded that temperature did not have significant influence on production of these compounds, for liquid medium. The same was observed for samples of liquid medium, with 5 days of growth, at 25 and 37 °C. In solid medium, the results showed that samples at 25 °C, with 3 and 5 days of growth did not have significant differences ($p > 0.05$).

In solid medium, it was possible to observe that the production of metabolites was affected by temperature. Between the samples with 3 and 5 days of growth at 37 °C, significant differences ($p < 0.05$) were found.

Furthermore, it was possible to observe three similar growth conditions: solid medium at 25 °C for 3 and 5 days, liquid medium for 3 days at 37 °C. Between these conditions, significant differences ($p > 0.05$) were not found. Also, since there were no significant differences ($p > 0.05$) between 3 and 5 days of growth at 25 °C, in solid medium.

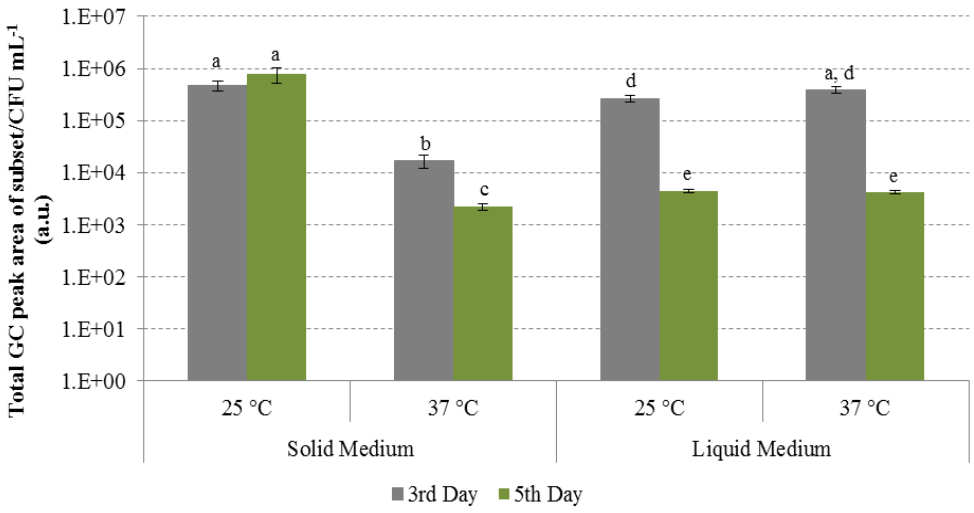

Figure 13 - GC peak area normalized by CFU mL^{-1} of a subset of 44 metabolites identified for *A. niger*. Each column represents the average calculated for three replicates and error bars represent the standard deviation. Different letters (a-e) represent significant differences ($p < 0.05$) among conditions.

The PCA was also applied to this subset of 44 metabolites. Considering this subset of 44 metabolites, there was a reduction of dataset complexity. PCA score plot has shown separation between the tested conditions with PC1 and PC2 explaining 47.8 % of the variability of the subset. The samples dispersed along PC1, depending on the type of medium, being liquid medium located on PC1 positive and solid medium located on PC1 negative.

The culture medium has impact in *A. niger* metabolism. In solid medium was observed an increase of specificity between 25 and 37 °C and allowed a clusters formation.

Based on PC1 and PC2 loadings plot (Figure 14-B), 16 metabolites contributed for the variability of samples from solid medium, being these metabolites: 1-octen-3-ol; 3-octanol; 2-ethyl-1-hexanol; 1-octanol; 2,4-bis(1,1-dimethylethyl)-phenol; hexanal; 2-undecenal; methyl 2-methylpropenoate; hexadecane; 1-methyl-2-(1-methylethyl)benzene; 2-methyl-6-phenyl-1,6-heptadiene; 3-heptanone; 6-methyl-5-hepten-2-one; 6,10-dimethyl-5,9-undecadien-2-one and endobornyl acetate. In terms of samples from liquid medium, the metabolites that contributed for their variability were: 1- butanol; 3-methyl-1-butanol; 1-hexanol; 1-heptanol; 2,6-dimethyl-7-octen-2-ol; 2-phenylethanol; 3-methylbutanal; heptanal; nonanal; decanal; dodecanal; benzaldehyde; benzeneacetaldehyde; 3-hydroxy-2,4,4-trimethylpentyl 2-methyl-propanoate; 2-phenylethyl acetate; heptadecane; benzene; toluene; 1,3-dimethylbenzene; 1,2-dimethylbenzene; propylbenzene; 1-ethyl-4-

methylbenzene; 1,3,5-trimethylbenzene; 1,2,3-trimethylbenzene; 2-ethyl-1,4-dimethylbenzene; biphenyl; 2-propanone; 3-nonen-2-one; and α-iso-methyl ionone.

Within the number of 504 compounds identified, 44 of them were found in several growth conditions.

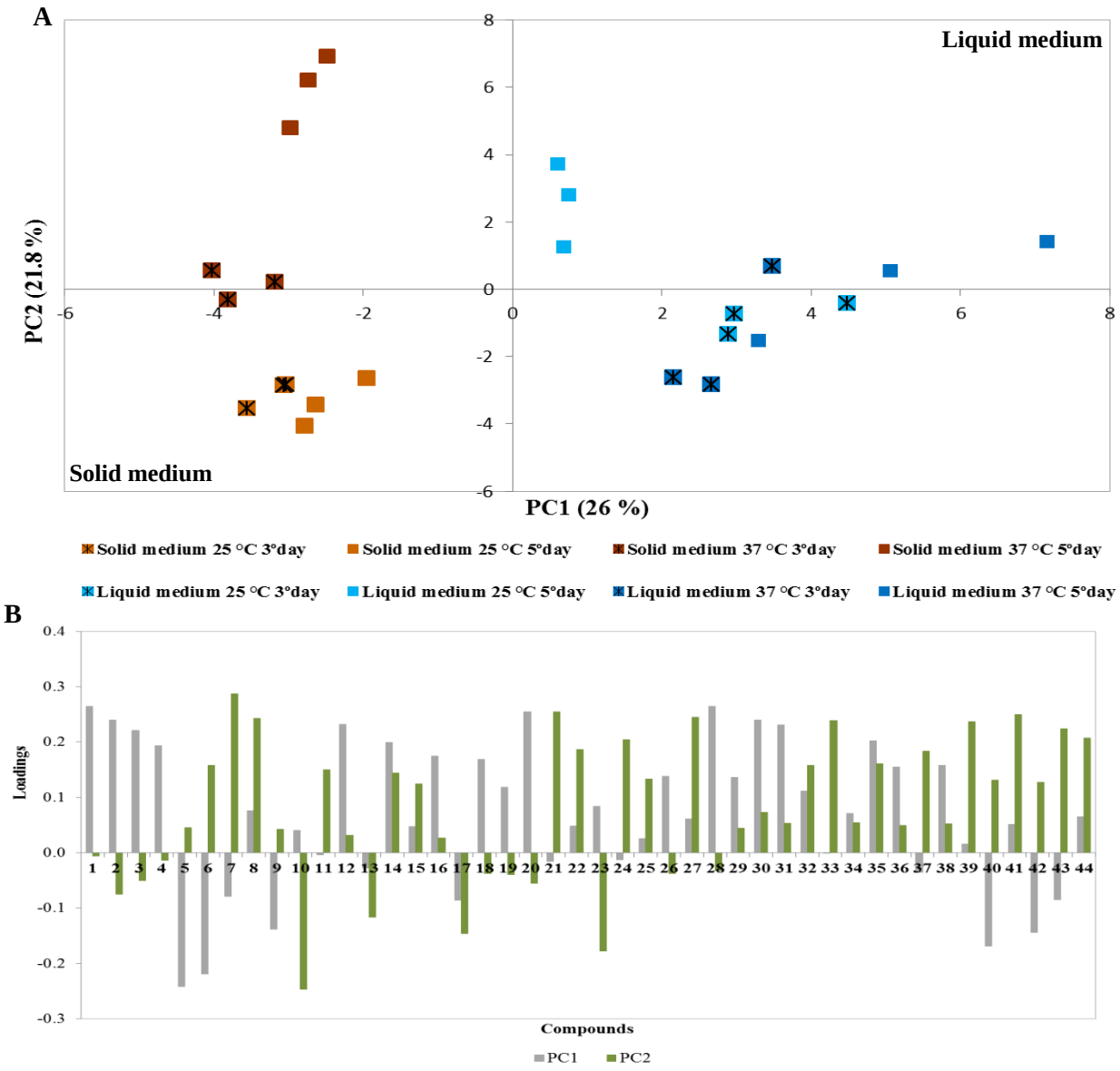

Figure 14 - A - PCA scores plot based on GC×GC peak areas of a subset of 44 metabolites. **B** - PC1 and PC2 loadings plot explaining the separation observed in scores map. The variables are organized according subset of 44 metabolites. The numbers represent the 44 metabolites: **1**- 1- Butanol; **2**- 3-Methyl-1-butanol; **3**- 1-Hexanol; **4**- 1-Heptanol; **5**- 1-Octen-3-ol; **6**- 3-Octanol; **7**- 2-Ethyl- 1-hexanol; **8**- 2,6-Dimethyl-7-octen-2-ol; **9**- 1- Octanol; **10**- 2-Phenylethanol; **11**- 2,4-bis(1,1-dimethylethyl)-phenol; **12**- 3-Methylbutanal; **13**- Hexanal; **14**- Heptanal; **15**- Nonanal; **16**- Decanal; **17**- 2-Undecenal; **18**- Dodecanal; **19**- Benzaldehyde; **20**- Benzeneacetaldehyde; **21**- Methyl 2-methylpropenoate; **22**- 3-hydroxy-2,4,4-trimethylpentyl 2-methyl-propanoate; **23**- 2-Phenylethyl acetate; **24**- Hexadecane; **25**- Heptadecane; **26**- Benzene; **27**- Toluene; **28**- 1,3-Dimethylbenzene; **29**- 1,2-Dimethylbenzene; **30**- Propylbenzene; **31**- 1-Ethyl-4-methylbenzene; **32**- 1,3,5- Trimethylbenzene; **33**- 1-Methyl-2-(1-methylethyl)benzene; **34**- 1,2,3-Trimethylbenzene; **35**- 2-Ethyl-1,4-dimethylbenzene; **36**- Biphenyl; **37**- 2-Methyl-6-phenyl-1,6-heptadiene; **38**- 2-Propanone; **39**- 3-Heptanone; **40**- 6-Methyl-5-hepten-2-one; **41**- 3-Nonen-2-one; **42**- 6,10-Dimethyl-,5,9-undecadien-2-one; **43**- Endobornyl acetate; **44**- α-iso-methyl ionone.

4. Concluding remarks

This study demonstrates the applicability of the HS-SPME/GC×GC–ToFMS methodology for the volatile compounds identified from *A. niger* cultures in YGC medium. In total were detected around 600-700 compounds per sample. It was possible to identify 504 metabolites from wide range of chemical families. Nevertheless, the YGC medium have influence in these samples, and for that reason, compounds from some chemical families were eliminated once they are present in higher level in the growth medium, compared to the samples with *A. niger*. Thus, the dataset was reduced, being comprised by 430 metabolites, and these compounds indicate the high complexity of *A. niger* exometabolome and also can characterize the metabolomic profile of *A. niger*. The major chemical families from exometabolome were alcohols, aldehydes, esters, hydrocarbons, ketones and terpenic compounds. A subset of 44 metabolites present in the *A. niger* cultures, independently of the growth conditions, was assembled over several chemical families, such as alcohols, aldehydes, esters, hydrocarbons, ketones, terpenic compounds and C_{13} norisoprenoids. These compounds could be considered as targeted metabolomic pattern of *A. niger*.

Chapter 3: Exploring the metabolomic profile of *Aspergillus niger*: Comparison with *Penicillium chrysogenum* and *Candida albicans*

Chapter 3: Exploring the metabolomic profile of *Aspergillus niger*: Comparison with *Penicillium chrysogenum* and *Candida albicans*

1. Introduction

The main causes of invasive fungal infections (IFI) are filamentous fungi as *Aspergillus* spp. and yeasts as *Candida* species. These microorganisms represent a major cause of increased morbidity and mortality in critically ill patients (Sipsas & Kontoyiannis, 2012). *Aspergillus* species can cause invasive aspergillosis, which is a major cause of morbidity and mortality in immunocompromised patients (Dinand, *et al.*, 2013, Ergene, *et al.*, 2013). Invasive aspergillosis (IA) appears to be an opportunistic infection and can involve multiple organs. Fungal infection mortality rate is high, which strongly suggests the need for prevention or earlier diagnosis and treatment (Badiee, *et al.*, 2011). This infection is caused by different *Aspergillus* spp., which invade lung tissue through the respiratory tract, enter the bloodstream and can disseminate to other organs (Pan, *et al.*, 2011). *Aspergillus niger*, as well as other *Aspergillus* species, produces a mycotoxin denominated ochratoxin A. Its occurrence has been reported in food and beverages, for example in grapes and their derived products (Chiotta, *et al.*, 2013). *Candida albicans* is a human commensal fungus that can be isolated from the gastrointestinal tract, oral cavity and vaginal mucosa (Kim & Sudbery, 2011). This yeast can be isolated from approximately 70% from healthy population and it is the fourth leading cause of bloodstream infections. In immunocompromised patients, *C. albicans* contributes from 37-44% of mortality (Han, *et al.*, 2011). Invasive fungal infection by *C. albicans* is very common and is a major problem in the intensive care unit. The candidaemia is an infection of the bloodstream caused by *Candida* species and it has a mortality rate up 50%, being associated with an increase in hospitalization and prolonged mechanical ventilation (Dhillon & Clark, 2011). *Penicillium* species occur worldwide and can cause destructive rots in the food industry, where they produce a wide range of mycotoxins. It has a large economic impact for population (Visagie, *et al.*, 2014). *Penicillium chrysogenum* is one of the most commonly occurring *Penicillium* species in indoor air (Fischer, *et al.*, 2003).

Microbial volatile organic compounds result of the primary and secondary metabolism of microorganisms and they are detectable before any visible sign of microbial growth. For this reason, they can be used as early indicators of the presence of microorganisms (Polizzi, *et al.*, 2012). They provide information regarding the state of biological organisms which can be used as a diagnostic tool for diseases through the detection of fungal specific metabolites pattern. Thus, this Chapter aims to evaluate the

applicability of the subset of metabolites establish on Chapter 2 (Table 2) from *A. niger* exometabolome to compare with others fungi, namely a yeast - *C. albicans* and a filamentous fungus – *P. chrysogenum*. A methodology based on headspace-solid phase microextraction combined with comprehensive two-dimensional gas chromatography coupled to mass spectrometry with a high resolution time of flight analyser (HS-SPME/GC×GC-ToFMS) was used. The subset was analysed in different growth conditions: at 25 °C, solid YGC medium, during 3 and 5 days. According to a previous study, the solid medium was selected due to the relative abundance of compounds was greater and the metabolites peaks have a higher intensity. At 25 °C, each metabolite peak had higher intensity when compared with 37 °C and the 3 days of growth are considered important once it allows a rapid detection of fungal infections, comparing with 5 days. These conditions are similar to the conditions used in clinical context, namely, solid medium at 25 °C. In order to validate the robustness of data, the chromatographic areas of *A. niger* metabolites present in growth conditions above mentioned (Chapter 2) were used. Supervised multivariate analysis Partial Least Squares Discriminant Analysis (PLS-DA) was applied to the selected subset of metabolites.

2. Material and methods

2.1. Fungal strain

For this study, a strain of *A. niger*, *P. chrysogenum* and *C. albicans* were used. *A. niger* and *P. chrysogenum* were provided by the Applied and Environmental Laboratories of the Biology and *C. albicans* was provided by Laboratory of RNA Biology from the Biology Department of Aveiro University. The initial culture of *A. niger* and *P. chrysogenum* were inoculated in Yeast Glucose Chloramphenicol Agar (YGC, Liofilchem®) and incubated at 25 °C, for 7 days, in order to obtain isolated colonies for later use in the preparation of new cultures in solid medium. *C. albicans* was inoculated in Yeast Glucose Chloramphenicol Agar (YGC, Liofilchem®) and incubated at 25 °C, for 48 h.

2.2. Culture conditions and preparation of samples

The experimental assays were carried out under different conditions. The *A. niger*, *P. chrysogenum* and *C. albicans* were incubated in different growth conditions: culture medium (solid YGC), temperature (25 °C) and incubation time (3 and 5 days). Three independent assays were performed for each condition.

The samples incubated in solid medium, were added of 10 mL of Ringer solution (Merck Millipore) per plate (5 plates). Then a loopful was used to help to remove the cellular content. Twenty-five millilitres of each sample were collected for exometabolome analysis and twenty-five millilitres for the determination of cell concentration.

2.3. Determination of cell concentration

The determination of cell concentration was obtained through the colony forming-units (CFU) counts. The samples were homogenized by means of mashing. Culture aliquots were serially diluted in Ringer solution and 100 µL of each diluted sample were plated on YGC, (five replicates per dilution). The plates of *A. niger* and *P. chrysogenum* were incubated at 25 °C for 7 days; the plates of *C. albicans* were incubated at 25 °C for 48 hours. The concentration of viable cells was expressed in colony forming-units per millilitre (CFU mL^{-1}). These results were used for the normalization of total areas of compounds obtained from exometabolome analysis.

2.4. Determination of exometabolome

2.4.1. Samples preparation

After incubation, for each sample, 25 mL of culture were collected and centrifuged at 10 000 rpm, at 4 °C, for 15 min (Centrifuge Beckman AVANTI). For headspace solid-phase microextraction (HS-SPME) assay 20 mL (1/β ratio of 0.5) of supernatant were transferred into a 60 mL glass vial, through syringe and syringe filter with a pore 0.20 µm. After the addition of 4 g of NaCl (≥99.5%; Sigma-Aldrich) and stirring bar of 20×5 mm, the vial was capped with a polytetrafluoroethylene septum and an aluminium cap (Chromacol Ltd., Herts, UK). The samples were stored at -80 °C until further analysis.

2.4.2. Metabolites determination

The methodology for the metabolites determination was performed through HS-SPME/GC×GC-ToFMS, according to the procedure described in the Chapter 2 of this master thesis.

2.4.3. Data processing

A full dataset comprises 44 metabolites which were previously considered the targeted metabolomic pattern of *A. niger*. A subset of 16 metabolites was also established by the compounds identified by GC×GC–ToFMS, and were achieved using Variable Importance in Projection (VIP).

For discrimination of the different microorganisms, GC peak areas of the subset of selected metabolites were used to build the data matrix for multivariate analysis. This matrix consisted of 30 observations: 2 species (*C. albicans* and *P. chrysogenum*), each one corresponding to 2 growth conditions, and 3 independent replicates, and one more specie (*A. niger*), corresponding also to 2 growth conditions, and with 9 independent replicates (from those, 3 replicates were obtained from original data of Chapter 2) and 44 or 16 variables (GC peak areas from the compounds in table 3).

Classification model for recognition of three species was calculated using PLS-DA. Peak areas were normalized by the total area, mean centered and autoscaled prior to calculations, in order to give to variables the same weight. The classification model complexity (number of latent variables) of the full dataset (44 metabolites) was computed,

as well as classification rate and Q^2 (quality-of-fit criterion) were estimated by cross-validation. Model robustness was assessed using permutation test with 1000 permutations. PLS-DA, permutation test and VIP algorithms were implemented in MATLAB, v. 7.12 (release 2011a).

3. Results and discussion

In the previous Chapter of this master thesis, it was established a subset of 44 compounds (Table 2) that were produced by *A. niger*, in all tested growth conditions. Then, this target metabolomic pattern of *A. niger* was compared with others microorganisms, such as *C. albicans* and *P. chrysogenum*, in order to explore the influence of the produced volatile compounds and the growth time (3 and 5 days). For this approach, a matrix was built according to the data available on Table 3, and then was applied PLS-DA to the normalized GC×GC chromatographic areas.

Table 3 has the GC peak areas registered for the samples under study, in which 3 different essays were performed for *A. niger* (the first column corresponds to the data from Chapter 2, and a difference of four orders of magnitude was observed between this dataset and two others performed essays) and one essay for the others microorganisms.

The microorganisms in this study are all fungi, being their metabolic pathways very similar (KEGG: Kyoto Encyclopedia of Genes and Genomes). Thus, most of this subset of 44 metabolites was present in the three species under study. The differences registered were: 1-heptanol was not present in *P. chrysogenum* for 3 and 5 days of growth, while for *C. albicans*, 1-butanol and 3-nonen-2-one were not present in cultures with 5 days of growth.

Furthermore, it is possible to observe that GC peak areas from some compounds (examples: 1-octen-3-ol, 3-octanol, 2-undecenal, nonanal) have similar values for *A. niger* and *P. chrysogenum*, when comparing with *C. albican*s. Actually *A. niger* and *P. chrysogenum* are filamentous fungi, while *C. albicans* is a yeast, thus it is likely that both filamentous fungi will have more resemblance with each other.

Table 3 – GC peak areas and respective RSD for the subset of 44 compounds detected for several microorganisms (*A. niger*, *C. albicans*, *P. chrysogenum*) at 3 and 5 days of growth, at 25 ºC in solid medium.

Peak number	Compound	3rd day					5th day				
		*A. niger**	*A. niger*	*A. niger*	*C. albicans*	*P. chrysogenum*	*A. niger**	*A. niger*	*A. niger*	*C. albicans*	*P. chrysogenum*
		Peak area[b] ($x10^{-2}$) and RSD (%)									
	Alcohols										
	Aliphatics										
14	1-Butanol	6.06 (24)	3.16 (60)	1.42 (36)	0.55 (36)	2.04 (31)	2.55 (38)	0.21 (31)	0.20 (41)	-	0.01 (59)
18	3-Methyl-1-butanol	429.19 (27)	85.92 (27)	48.46 (20)	79.30 (14)	6.31 (18)	1047.35 (39)	0.20 (56)	0.15 (44)	5.30 (45)	0.73 (60)
27	1-Hexanol	2.84 (16)	14.26 (21)	11.31 (17)	0.06 (16)	4.09 (24)	2.19 (41)	0.36 (17)	0.13 (50)	0.09 (25)	0.03 (34)
37	1-Heptanol	4.62 (22)	11.77 (28)	13.04 (14)	0.32 (35)	-	6.81 (36)	0.53 (30)	0.61 (38)	0.14 (94)	-
39	1-Octen-3-ol	1218.13 (17)	11044.33 (83)	13865.75 (18)	0.23 (23)	11114.95 (40)	1087.31 (19)	269.13 (41)	138.64 (80)	0.26 (4)	94.35 (67)
44	3-Octanol	888.57 (25)	4234.87 (44)	8784.41 (67)	0.16 (38)	845.41 (31)	809.07 (30)	170.54 (23)	339.22 (93)	0.95 (43)	48.56 (5)
46	2-Ethyl-1-hexanol	50.38 (30)	788.11 (7)	655.02 (12)	16.54 (14)	896.45 (20)	150.51 (47)	30.56 (25)	29.62 (27)	8.27 (11)	13.99 (2)
49	2,6-Dimethyl-7-octen-2-ol	6.51 (43)	55.39 (20)	117.12 (89)	1.23 (40)	44.62 (22)	12.30 (45)	3.08 (47)	2.46 (36)	1.42 (129)	0.86 (18)
52	1-Octanol	24.78 (38)	65.60 (63)	60.87 (51)	0.42 (26)	714.69 (12)	48.15 (38)	5.35 (57)	3.84 (29)	0.41 (1)	6.67 (15)
	Aromatics										
79	2-Phenylethanol	1199.03 (48)	18.78 (39)	10.88 (47)	95.88 (37)	44.70 (84)	3198.84 (48)	0.10 (60)	1.40 (75)	65.95 (17)	0.03 (64)
83	2,4-bis(1,1-dimethylethyl)-phenol	23.78 (22)	808.03 (20)	618.25 (7)	11.30 (5)	595.30 (30)	96.43 (41)	10.15 (32)	18.62 (36)	2.59 (9)	6.59 (42)
	Aldehydes										
	Aliphatics										
89	3-Methylbutanal	18.93 (42)	20.04 (16)	19.67 (15)	1.12 (21)	136.97 (21)	36.83 (37)	1.14 (25)	1.16 (18)	0.23 (16)	0.18 (14)
95	Hexanal	46.41 (20)	60.45 (19)	51.89 (6)	0.60 (39)	46.20 (53)	46.77 (18)	1.65 (9)	1.59 (24)	0.34 (22)	0.38 (63)
97	Heptanal	13.38 (29)	60.31 (14)	64.52 (4)	1.18 (26)	30.00 (22)	18.27 (29)	1.35 (18)	1.41 (28)	0.36 (10)	0.54 (44)
107	Nonanal	144.30 (28)	501.84 (7)	481.86 (12)	7.73 (24)	1028.06 (19)	180.11 (35)	15.82 (38)	11.25 (18)	5.43 (9)	14.28 (37)
110	Decanal	93.75 (38)	36.91 (59)	28.29 (79)	9.22 (50)	850.08 (10)	125.35 (44)	7.89 (21)	4.61 (13)	3.98 (5)	1.71 (31)
116	2-Undecenal	20.50 (25)	27.97 (64)	23.08 (20)	0.36 (8)	31.03 (24)	254.25 (40)	1.04 (36)	1.03 (20)	0.19 (20)	0.17 (21)
117	Dodecanal	11.52 (39)	158.65 (44)	131.30 (17)	4.36 (31)	425.66 (13)	46.27 (53)	0.87 (18)	1.03 (35)	1.10 (5)	1.65 (29)
	Aromatics										
121	Benzaldehyde	142.07 (26)	1073.31 (17)	872.78 (16)	6.17 (15)	259.78 (26)	147.46 (33)	21.38 (28)	30.53 (15)	3.14 (32)	2.56 (23)
122	Benzeneacetaldehyde	37.91 (41)	39.98 (25)	57.14 (4)	0.76 (12)	49.97 (54)	55.01 (48)	0.86 (27)	0.98 (25)	0.48 (17)	0.37 (16)
	Esters										
	Aliphatics										
134	Methyl 2-methylpropenoate	2.70 (16)	4.96 (46)	4.40 (11)	0.06 (43)	4.77 (17)	16.01 (28)	0.09 (28)	0.13 (15)	0.02 (17)	0.04 (18)
171	3-hydroxy-2,4,4-trimethylpentyl 2-methyl-propanoate	18.20 (50)	217.21 (25)	228.34 (16)	1.89 (39)	437.35 (27)	38.91 (44)	4.63 (15)	4.88 (31)	0.76 (19)	2.84 (33)
	Aromatics										
187	2-Phenylethyl acetate	70.90 (16)	2.02 (47)	0.83 (26)	0.14 (11)	2.87 (44)	95.21 (44)	0.03 (7)	0.06 (75)	0.05 (43)	0.01 (8)
	Hydrocarbons										
	Aliphatics										
283	Hexadecane	2.16 (26)	26.88 (27)	27.11 (30)	0.26 (12)	28.50 (27)	4.17 (36)	0.52 (17)	0.79 (27)	0.11 (11)	0.48 (34)
284	Heptadecane	4.06 (38)	25.16 (16)	25.86 (25)	0.81 (43)	23.53 (52)	6.63 (41)	0.66 (63)	1.13 (30)	0.16 (66)	0.11 (50)
	Aromatics										
287	Benzene	2.73 (29)	5.02 (23)	3.85 (11)	0.10 (38)	6.87 (36)	3.05 (28)	0.22 (66)	0.23 (45)	0.03 (13)	0.04 (38)
288	Toluene	8.87 (19)	773.85 (14)	504.99 (49)	2.60 (69)	225.58 (45)	39.47 (43)	17.14 (35)	37.84 (38)	4.62 (9)	4.85 (30)
290	1,3-Dimethylbenzene	5.84 (29)	55.41 (18)	41.55 (10)	1.23 (29)	16.71 (17)	7.45 (29)	0.52 (11)	1.96 (36)	0.24 (23)	0.29 (32)
291	1,2-Dimethylbenzene	2.28 (46)	22.85 (34)	17.06 (32)	0.39 (50)	14.36 (47)	4.12 (22)	0.60 (37~)	0.87 (33)	0.06 (25)	0.23 (58)
294	Propylbenzene	0.32 (26)	4.31 (22)	2.90 (2)	0.06 (52)	3.40 (16)	0.95 (14)	0.08 (42)	0.09 (13)	0.03 (85)	0.02 (15)
296	1-Ethyl-4-methylbenzene	0.84 (47)	14.82 (46)	11.32 (44)	0.13 (72)	10.04 (51)	5.55 (51)	0.22 (51)	0.27 (28)	0.04 (89)	0.04 (24)
299	1,3,5-Trimethylbenzene	3.23 (37)	56.90 (28)	41.90 (30)	0.44 (78)	17.24 (2)	24.93 (23)	1.54 (45)	1.58 (60)	0.34 (84)	0.05 (8)
301	1-Methyl-2-(1-methylethyl)benzene	0.95 (25)	5.92 (33)	5.08 (8)	0.10 (86)	4.79 (26)	1.27 (54)	0.21 ((42)	0.28 (15)	0.04 (12)	0.06 (36)
302	1,2,3-Trimethylbenzene	0.79 (64)	16.16 (35)	13.11 (20)	0.11 (51)	16.78 (24)	5.02 (47)	0.32 (47)	0.44 (22)	0.08 (71)	0.12 (58)
304	2-Ethyl-1,4-dimethylbenzene	0.24 (39)	2.84 (33)	3.37 (45)	0.01 (91)	4.82 (64)	0.76 (16)	0.09 (60)	0.13 (16)	-	0.03 (8)
314	Biphenyl	1.61 (47)	9.35 (22)	9.54 (19)	0.14 (14)	9.66 (30)	2.84 (21)	0.32 (56)	0.42 (25)	0.11 (37)	0.15 (7)
316	2-Methyl-6-phenyl-1,6-heptadiene	3.58 (35)	670.44 (31)	590.73 (12)	6.10 (96)	877.31 (67)	7.57 (40)	21.40 (15)	23.92 (20)	3.40 (11)	5.94 (5)
	Ketones										
	Aliphatics										
335	2-Propanone	23.79 (18)	129.98 (19)	102.81 (7)	0.66 (15)	90.51 (40)	40.52 (40)	3.12 (20)	3.17 (18)	0.33 (2)	1.10 (25)
348	3-Heptanone	0.70 (30)	9.68 (14)	7.60 (13)	0.02 (5)	12.10 (3)	0.60 (38)	0.19 (31)	0.25 (31)	0.03 (5)	0.35 (12)
353	6-Methyl-5-hepten-2-one	46.66 (23)	100.49 (35)	79.45 (34)	2.57 (22)	26.38 (8)	30.33 (35)	4.75 (4.75)	8.11 (43)	1.60 (29)	1.15 (18)
364	3-Nonen-2-one	0.79 (41)	3.85 (67)	1.88 (47)	0.11 (37)	3.01 (21)	0.92 (23)	0.33 (28)	0.45 (57)	-	0.09 (40)
382	6,10-Dimethyl-,5,9-undecadien-2-one	44.58 (52)	435.50 (20)	485.10 (13)	3.01 (28)	530.07 (24)	55.35 (29)	15.01 (45)	21.42 (28)	1.99 (19)	6.95 (30)
	Terpenic compounds										
484	Endobornyl acetate	9.34 (50)	104.61 (59)	118.27 (57)	0.81 (14)	66.38 (42)	12.37 (40)	5.12 (42)	3.17 (75)	0.23 (96)	1.60 (30)
504	α-iso-methyl ionone	2.71 (39)	19.26 (35)	28.94 (36)	0.23 (6)	36.57 (27)	4.36 (22)	0.76 (53)	0.90 (25)	0.11 (17)	0.57 (11)

*GC peak areas from this study are multiplied by 10^2

Figure 15 shows the scores and loadings scatter plot of PLS-DA regarding the distinction of *A. niger*, *P. chrysogenum* and *C. albicans*. LV1 and LV2 explain *ca.* 37 % of the variability of the dataset (Figure 15A). The *C. albicans* samples are dispersed throughout LV2 positive, while filamentous fungi are dispersed in LV2 negative. A separation of the filamentous fungi is obtained by LV1 axis, where *P. chrysogenum* and *A. niger* samples are dispersed throughout the LV1 negative and LV1 positive, respectively. Thus, a good discrimination between samples classes (different microorganisms) was observed, nevertheless, the differences between grown times smaller compared to the differences between three microorganisms under study.

Different compounds contributed to the distinction of the three microorganisms under study (Figure 15B). Some metabolites, such as 1- butanol; 3-methyl-1-butanol; 1-hexanol; 1-octen-3-ol; 3-octanol; 1-heptanol; 2-phenylethanol; hexanal; 2-undecenal; benzaldehyde; benzeneacetaldehyde; methyl 2-methylpropenoate; 2-phenylethyl acetate; benzene; 1,3,5- trimethylbenzene; 6-methyl-5-hepten-2-one, contributed for the variability of *A. niger* samples. While the variability of the samples of *C. albicans* can be derived from the contribution of 2-ethyl- 1-hexanol; 2,6-dimethyl-7-octen-2-ol; 2,4-bis(1,1-dimethylethyl)-phenol; 3-methylbutanal; heptanal; decanal; dodecanal; heptadecane; 1,3-dimethylbenzene; 1,2-dimethylbenzene; propylbenzene; 1-ethyl-4-methylbenzene; 1-methyl-2-(1-methylethyl)benzene; biphenyl. In terms of *P. chrysogenum*, the compounds that contributed were: 1- octanol; nonanal; 3-hydroxy-2,4,4-trimethylpentyl 2-methyl-propanoate; hexadecane; toluene; 1,2,3-trimethylbenzene; 2-ethyl-1,4-dimethylbenzene; 2-methyl-6-phenyl-1,6-heptadiene; 2-propanone; 3-heptanone; 3-nonen-2-one; 6,10-dimethyl-,5,9-undecadien-2-one; endobornyl acetate; α-iso-methyl ionone.

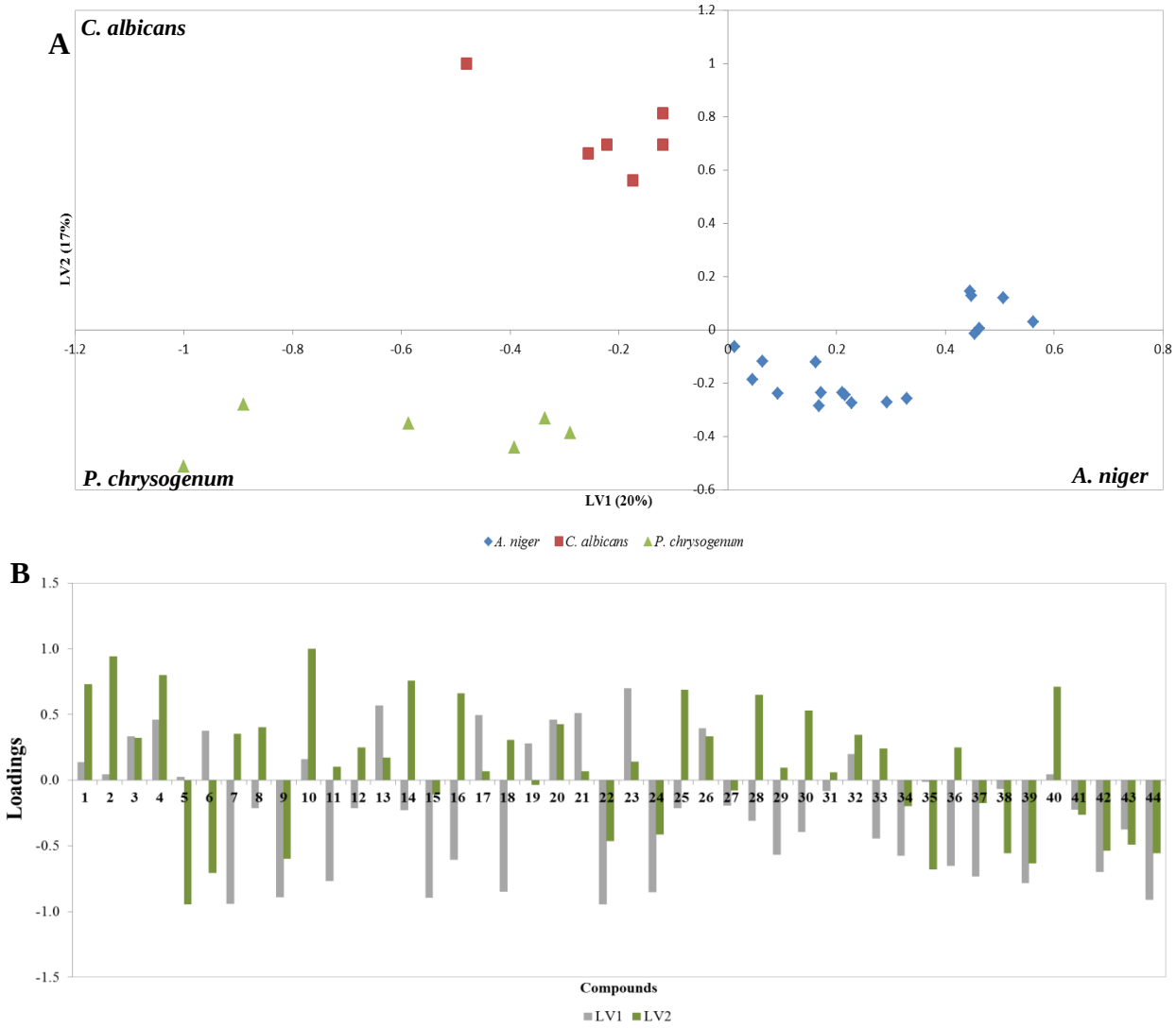

Figure 15 A - PLS-DA scores plot applied to GC×GC peak areas of a subset of 44 metabolites. **B** - LV1 and LV2 loadings plot explaining the separation observed in scores map. The variables are organized according subset of 44 metabolites.

The numbers represent the 44 metabolites: **1**- 1- Butanol; **2**- 3-Methyl-1-butanol; **3**- 1-Hexanol; **4**- 1-Heptanol; **5**- 1-Octen-3-ol; **6**- 3-Octanol; **7**- 2-Ethyl- 1-hexanol; **8**- 2,6-Dimethyl-7-octen-2-ol; **9**- 1- Octanol; **10**- 2-Phenylethanol; **11**- 2,4-bis(1,1-dimethylethyl)-phenol; **12**- 3-Methylbutanal; **13**- Hexanal; **14**- Heptanal; **15**- Nonanal; **16**- Decanal; **17**- 2-Undecenal; **18**- Dodecanal; **19**- Benzaldehyde; **20**- Benzeneacetaldehyde; **21**- Methyl 2-methylpropenoate; **22**- 3-hydroxy-2,4,4-trimethylpentyl 2-methyl-propanoate; **23**- 2-Phenylethyl acetate; **24**- Hexadecane; **25**- Heptadecane; **26**- Benzene; **27**- Toluene; **28**- 1,3-Dimethylbenzene; **29**- 1,2-Dimethylbenzene; **30**- Propylbenzene; **31**- 1-Ethyl-4-methylbenzene; **32**- 1,3,5- Trimethylbenzene; **33**- 1-Methyl-2-(1-methylethyl)benzene; **34**- 1,2,3-Trimethylbenzene; **35**- 2-Ethyl-1,4-dimethylbenzene; **36**- Biphenyl; **37**- 2-Methyl-6-phenyl-1,6-heptadiene; **38**- 2-Propanone; **39**- 3-Heptanone; **40**- 6-Methyl-5-hepten-2-one; **41**- 3-Nonen-2-one; **42**- 6,10-Dimethyl-,5,9-undecadien-2-one; **43**- Endobornyl acetate; **44**- α-iso-methyl ionone.

PLS-DA analysis was validated through the permutation test, and was evaluated the classification model, where a class membership prediction was applied. The prediction error measure Q^2, which is the default parameter used in PLS-DA discrimination, focuses on how well the class label can be predicted from new dataset. This classification analysis provided a model with predictive Q^2 capability of 0.70 for *A. niger*, 0.68 for *C. albicans* and 0.80 for *P. chrysogenum*. Permutation test is the random change of classes (microorganisms) and calculation of classification models and it is integrated in the currently described validation procedure. This test allowed to evaluate the statistical significance of the model, i.e., it verifies if the classification obtained with the permuted model is similar to the initial model.

The initial model was statistically significant, the Q^2 value was 0.70 and all permuted models had a lower Q^2 value comparing with the initial model. This allowed to conclude that the initial model was not a random model. As an example, distribution of Q^2 values for the prediction of the *A. niger* class membership for the 1000 permutated models was showed in the (Figure 17) together with the Q^2 value for the initial model. Q^2 values for all permuted models were lower than the Q^2 for the initial model, indicating that the classification model is statistically significant ($p < 0.001$).

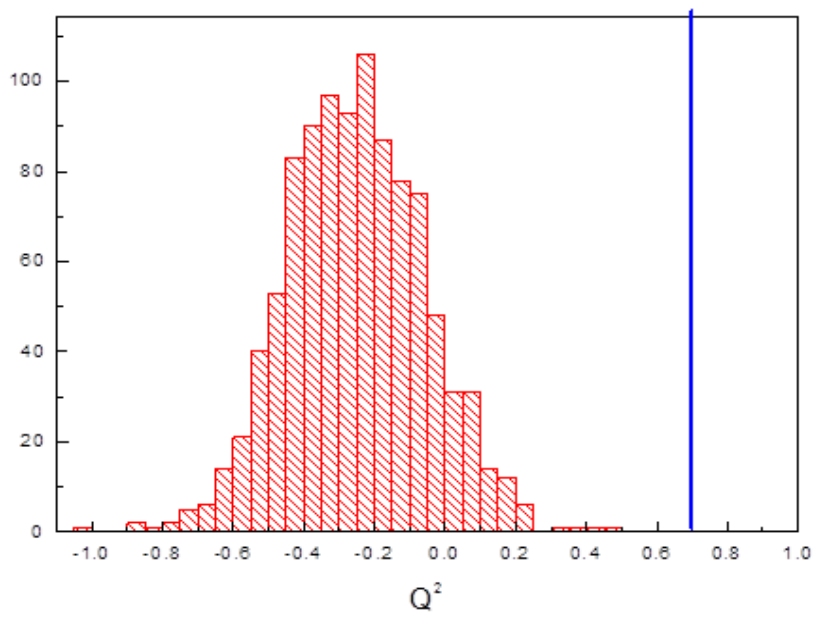

Figure 17 – Distribution of Q^2 values for the permuted models (1000 permutations) and Q^2 for the original model (blue line).

Table 3 indicates the metabolites with VIP (variable importance in the projection) value higher than 0.8, that were used as the dataset to perform a new PLS-DA analysis.

Table 3 - VIP values of PLS-DA of 44 metabolites.

VIP values	Metabolites
1.32	Dodecanal
1.31	Nonanal
1.25	1-Octanol
1.23	2-Ethyl-1-hexanol
1.18	Decanal
1.16	3-Octanol
1.04	3-Heptanone
1.04	α-iso-methyl ionone
1.04	3-hydroxy-2,4,4-trimethylpentyl 2-methyl-propanoate
0.96	Benzaldehyde
0.94	2-Phenylethanol
0.86	1-Octen-3-ol
0.85	1-Heptanol
0.84	2-Propanone
0.82	Hexadecane
0.81	3-Methyl-1-butanol

Based on the parameter VIP, the compounds that played important roles in the classification were chosen and the PLS-DA was recalculated. Variables selection allowed to decrease the number of parameters in the model and to improve classification model performance (Q^2 of 0.8 *vs.* 0.7 of the model comprising 44 metabolites). Therefore, with the decrease of 44 to 16 metabolites, the require analysis time was reduced.

Figure 18 shows the scores and loadings scatter plot of PLS-DA regarding the distinction of *A. niger*, *P. chrysogenum* and *C. albicans*, taking into account the VIP metabolites. LV1 and LV2 explain *ca.* 62 % of the variability of the dataset (Figure 18A). The discrimination between samples classes (different microorganisms) obtained had the same trend registered for the PLS-DA with 44 metabolites. Moreover, this PLS-DA analysis explained more variability of the dataset (62 %), when comparing with the PLS-DA with 44 metabolites (37 %).

The three microorganisms under study can be distinguish through the contribution of different compounds (Figure 18B). Some metabolites, such as 3-octanol and benzaldehyde can contribute for the samples variety of *A. niger*. While the variability of the samples of *C. albicans* can be contributed by 2-ethyl-1-hexanol; decanal; dodecanal. In

terms of *P. chrysogenum*, the compounds that contributed were: 1- octanol; 3-hydroxy-2,4,4-trimethylpentyl 2-methyl-propanoate; hexadecane; 2-propanone; 3-heptanone, and α-iso-methyl ionone.

The results clearly showed that this subset of metabolites had the power to distinguish *A. niger* from *P. chrysogenum* and *C. albicans*.

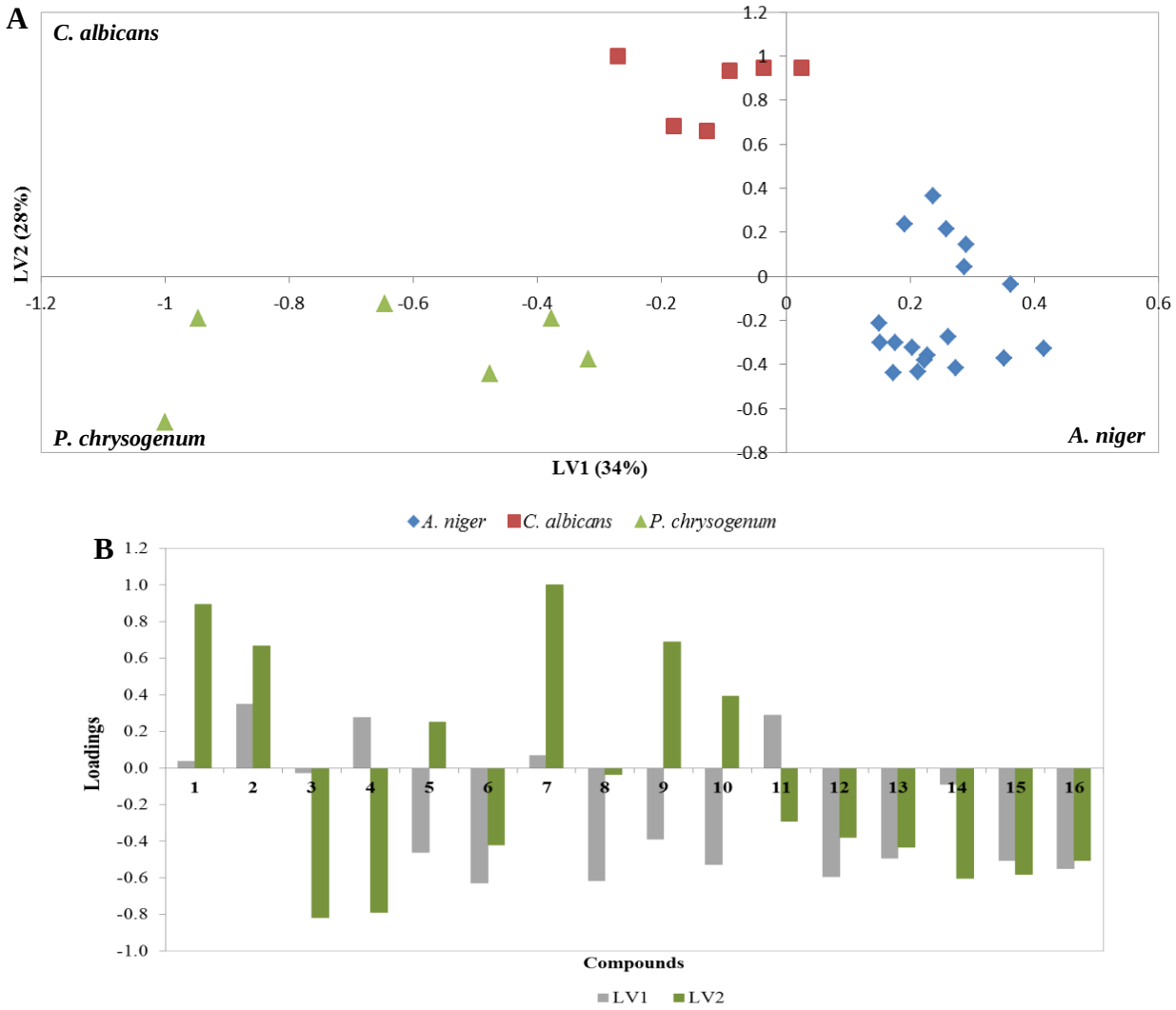

Figure 18 – A- PLS-DA scores plot applied to GC×GC peak areas of a subset of 16 metabolites. **B** - LV1 and LV2 loadings plot explaining the separation observed in scores map. The variables are organized according subset of 16 metabolites.
The numbers represent the 16 metabolites: **1-** 3-Methyl-1-butanol; **2-** 1-Heptanol; **3-** 1-Octen-3-ol; **4-** 3-Octanol; **5-** 2-Ethyl-1-hexanol; **6-** 1- Octanol; **7-** 2-Phenylethanol; **8-** Nonanal; **9-** Decanal; **10-** Dodecanal; **11-** Benzaldehyde; **12-** 3-hydroxy-2,4,4-trimethylpentyl 2-methyl-propanoate; **13-** Hexadecane; **14-** 2-Propanone; **15-** 3-Heptanone; **16-** α-iso-methyl ionone.

PLS model was validated using permutation test. To evaluate the classification model, a class membership prediction was applied. This classification analysis provided a model with predictive Q^2 capability of 0.86 for *A. niger*, 0.86 for *C. albicans* and 0.85 for *P. chrysogenum*.

The initial model was statistically significant, the Q^2 value was 0.85 and all the permuted models had a lower initial Q^2 value comparing with the initial model. This allowed to conclude that the initial model was not a random model. As an example, distribution of Q^2 values for the prediction of the *A. niger* class membership for the 1000 permutated models are represents in the Figure 19 together with the Q^2 value for the initial model. Q^2 values for all permuted models were lower than the Q^2 for the initial model, indicating that the classification model is statistically significant ($p < 0.001$).

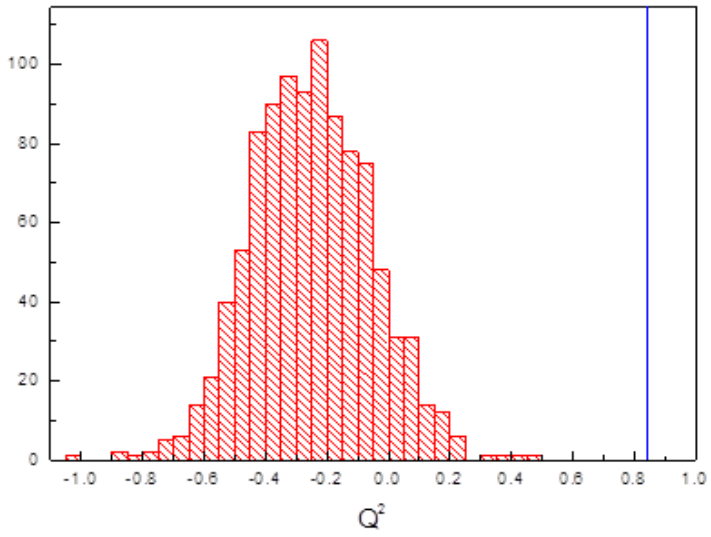

Figure 19- Distribution of Q^2 values for the permuted models (1000 permutations) and Q^2 for the initial model (blue line).

4. Concluding remarks

This study demonstrated the applicability of the HS-SPME/GC×GC–ToFMS methodology for distinguish the fungus *A. niger* from the others microorganisms, such as, *C. albicans* and *P. chrysogenum*, through a PLS-DA analysis. This PLS-DA analysis was based on a subset of 44 metabolites previously established (Chapter 2 of this master thesis).

PLS model was validated using class membership prediction and permutation test, which allowed to provide a statistically significant model with predictive Q^2 capability of 0.70 for *A. niger*, taking into account the initial 44 metabolites. Considering only the 16 metabolites from the VIP parameter, the obtained model had a predictive Q^2 capability of 0.86 for *A. niger*, which was significantly higher, being more robust than the previous.

These 16 metabolites and the predictive model obtained can be further explored in clinical context for the possible distinction of infected samples with the fungi studied in this master thesis. Furthermore, this subset of 16 metabolites allowed a reduction of the analysis time and the conditions used were similar to the conditions used in clinical context, solid medium, at 25 °C and *ca.* 1 week. However, in this study was possible to reduce the time for 3 days.

Chapter 4: Concluding remarks

Chapter 4: Conclusion

Aspergillus species is the second leading cause of fungal infection in hospitals and can cause invasive aspergillosis, which is the major cause of mortality in immunocompromised patients. These higher mortality rates could be due to the late diagnosis, which can lead to delays in the beginning of the antifungal therapy. Thus, this master thesis aims to establish the volatile exometabolome of *A. niger* by using advanced chromatographic technique and to search for molecular biomarker pattern that can be further exploited to fungal diagnosis. A methodology based on headspace-solid phase microextraction combined with comprehensive two-dimensional gas chromatography coupled to mass spectrometry with a high resolution time of flight analyser (HS-SPME/GC×GC-ToFMS) was applied.

In a first approach, it was used different growth conditions for the *A. niger*: growth time (3 and 5 days), temperature (25 and 37 °C) and culture medium (solid and liquid YGC). The application HS-SPME/GC×GC-ToFMS allowed the detection of 600-700 compounds per sample, from wide range of chemical families: acids, alcohols, aldehydes, esters, ethers, furan–type compounds, furanones, halogenated compounds, hydrocarbons, ketones, N-compounds, pyrazines, S-compounds, terpenic compounds and C_{13} norisoprenoid. From these, 504 metabolites were identified, however, taking into account the YGC medium composition, 74 compounds was removed from the original dataset. Thus, a reduced dataset comprises 430 metabolites, and they were considered for further studies, namely as *A. niger* exometabolome.

The GC×GC-ToFMS total ion chromatogram contour plot relative to *A. niger* samples grown in solid and liquid medium, was the snapshot of the volatile profile of *A. niger*. The solid medium at 25 °C showed the higher abundance of compounds. Also, another feature is that *A. niger* is a strictly aerobic microorganism, and its growth in liquid medium occurs at the surface, where oxygen is available. In liquid medium the available oxygen at 5 days of growth is lower, and less of GC peak area was detected, when comparing with GC peak area observed for 3 days of growth.

In order to have a targeted metabolomic pattern of *A. niger*, from the dataset of 430 metabolites, it was searched the metabolites that were always present in all growth conditions. Thus, a subset of 44 metabolites was assembled as volatile molecular biomarkers for *A. niger*. Considering this subset of 44 metabolites, there was a reduction of dataset complexity.

PCA was performed to observe the growth conditions impact in metabolism of *A. niger* for dataset of 430 and subset of 44 metabolites; the main results were that the different type of culture medium was dispersed along to PC1: liquid medium samples were localized in PC1, while solid medium samples were in PC2. For the dataset of 430 metabolites, the differences in volatile composition *A. niger* growth in liquid medium were contributed from acids, alcohols, ethers, hydrocarbons and ketones; while aldehydes and esters were contributed from the samples that were grown in solid medium. In conclusion, the culture medium has impact in *A. niger* metabolism.

Furthermore, this previous subset was compared with samples of *Candida albicans* (yeast) and *Penicillium chrysogenum* (filamentous fungi), and a distinction of the fungus *A. niger* was obtained from the others microorganisms, through a PLS-DA analysis. Permutation test was used to validate the PLS-DA model, where a statistically significant model with predictive Q^2 capability of 0.70 for *A. niger*, taking into account the initial 44 metabolites. When the subset of compounds were reduced to 16 (obtained by VIP parameter), the predictive Q^2 capability of the obtained model was 0.86 for *A. niger*, which was significantly higher, being more robust than the previous. The decrease of 44 to 16 metabolites, reduced the require analysis time and the conditions used were similar to the conditions used in clinical context, solid medium, at 25 °C and *ca.* 1 week. However, in this study was possible to reduce the time for 3 days.

In conclusion, the results allowed to conclude that the obtained subset of 44 metabolites can characterize *A niger*. They could be possibly applied as volatile molecular biomarkers in the detection of *A. niger* and distinguish this specie from others fungi for longer and shorter growth times.

Future perspectives:

Following this work, perform assays for application the developed methodology for diagnosis of fungal infections in clinical context;

- Test the developed methodology with different fungi concentrations;
- Infect blood or other biological samples with *A. niger* and test the limit of detection in GC×GC–ToFMS;
- Perform assays with clinical samples in order to test the applicability of metabolites pattern in detection of fungal infections caused by *Aspergillus niger*;

- Studies with other fungi with high clinical importance should be performed and a database of specific metabolomic pattern should be build, in order to allow the early diagnosis.

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Annexes

Table S1 - Volatile organic compounds produced by fungi, analyzed in several studies through different methods.

Metabolites	Microorganism	Method	Reference
	A: 1 2 3 4 5 6 7 8 9 10 11 12 13 14 · B C D E F G H: 1 2 · I · J: 1 2 3 4 5 6 7 8 9 10 11 12 · K: 1 2 · L · M · N		
Acids			
Acetic acid	K: + + · M: +	SPME/GC-MS	(Osorio-Cadavid, *et al.*, 2008)
Caprylic acid	K: + · M: +	SPME/GC-MS	(Osorio-Cadavid, *et al.*, 2008)
Phenylacetic acid	K: +	SPME/GC-MS	(Osorio-Cadavid, *et al.*, 2008)
Alcohols *Aliphatics*			
Ethanol	A: 6, 9, 14 · B: +, + · C: + · D: + · F: + · G: + · H: 1: + · H: 2: + · J: 9 · K: 1 2: + + · L: + · M: + · N: +	DHS/GC-MS HS/SIFT-MS HS-SPME/GC-MS SPME/GC-MS	(Caileux, *et al.*, 1992) (Scotter, *et al.*, 2005) (Fiedler, *et al.*, 2001) (Osorio-Cadavid, *et al.*, 2008)
1-Propanol	A: 6, 9, 14 · B: + · D: + · M: +	DHS/GC-MS	(Caileux, *et al.*, 1992)
Butanediol	H: 1: +	HS-SPME/GC-MS	(Fiedler, *et al.*, 2001)
1,3-Butanediol	K: + +	SPME/GC-MS	(Osorio-Cadavid, *et al.*, 2008)
2-Methyl-1-propanol	A: 9 · J: 9	SPME/GC-MS	(Matysik, *et al.*, 2008)
	A: 6, 9, 14 · B: + · D: + · M: +	DHS/GC-MS	(Caileux, *et al.*, 1992)
	A: 3, 6 · F: + · I: + · J: 1, 2, 4, 5, 6, 10	AE/GC-MS	(Fischer, *et al.*, 1999)
Furfuryl alcohol	K: + + · M: +	SPME/GC-MS	(Osorio-Cadavid, *et al.*, 2008)
2-Methyl-1-butanol	A: 3, 6, 14 · F: + · I: + · J: 1, 2, 4, 10	AE/GC-MS	(Fischer, *et al.*, 1999)
	A: 6 · H: 1: + · J: 9 · N: +	HS-SPME/GC-MS	(Fiedler, *et al.*, 2001)
	A: 9 · J: 2, 9	SPME/GC-MS	(Matysik, *et al.*, 2008)
	A: 9 · B: + · D: + · M: +	DHS/GC-MS	(Caileux, *et al.*, 1992)
3-Methyl-1-butanol	K: + + · M: +	SPME/GC-MS	(Osorio-Cadavid, *et al.*, 2008)
	A: 6, 14 · B: + · D: + · L: + · M: +	DHS/GC-MS	(Caileux, *et al.*, 1992)
	J: 7, 8, 11	HS-SPME/GC-MS	(Nilsson, *et al.*, 1996)
	A: 3, 6, 14 · F: + · J: 2, 4, 5, 10	AE/GC-MS	(Fischer, *et al.*, 1999)
	A: 6 · H: 1: + · J: 9 · N: +	HS-SPME/GC-MS	(Fiedler, *et al.*, 2001)
	A: 6, 9 · C: + · J: 2	SPME/GC-MS	(Matysik, *et al.*, 2008)
	A: 1, 3, 4, 5, 8, 9, 11	SPME/GC-MS	(Jelen & Grabarkiewicz-Szczesna, 2005)
3-Methyl-3-buten-1-ol	C: +	SPME/GC-MS	(Matysik, *et al.*, 2008)
1-Pentanol	K: +	SPME/GC-MS	(Osorio-Cadavid, *et al.*, 2008)
	C: + · J: 9	SPME/GC-MS	(Matysik, *et al.*, 2008)
2-Pentanol	A: 6, 9, 14 · B: + · C: + · J: 2, 9	SPME/GC-MS	(Matysik, *et al.*, 2008)
2-Ethyl-1-hexanol	A: 6, 14 · J: 9	SPME/GC-MS	(Matysik, *et al.*, 2008)
	A: 6 · H: 1: +	HS-SPME/GC-MS	(Fiedler, *et al.*, 2001)

Annexes

Metabolites	Microorganism																																					Method	Reference
	A															B	C	D	E	F	G	H		I	J													K		L	M	N	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14							1	2		1	2	3	4	5	6	7	8	9	10	11	12	1	2					
6-Methyl-heptanol						+																																		HS-SPME/GC-MS	(Fiedler, *et al.*, 2001)
1-Octen-3-ol			+											+						+				+																AE/GC-MS	(Fischer, *et al.*, 1999)
						+			+					+			+								+							+									SPME/GC-MS	(Matysik, *et al.*, 2008)
																																+		+								HS-SPME/GC-MS	(Nilsson, *et al.*, 1996)
						+			+	+				+											+							+									HS-SPME/GC-MS	(Fiedler, *et al.*, 2001)
	+	+		+	+		+	+	+		+	+	+																											SPME/GC-MS	(Jelen & Grabarkiewicz-Szczesna, 2005)
3-Octanol																														+				+							HS-SPME/GC-MS	(Nilsson, *et al.*, 1996)
						+			+					+			+								+							+									SPME/GC-MS	(Matysik, *et al.*, 2008)
									+																							+									HS-SPME/GC-MS	(Fiedler, *et al.*, 2001)
				+	+			+			+	+	+																												SPME/GC-MS	(Jelen & Grabarkiewicz-Szczesna, 2005)
2-Nonen-1-ol						+																																			SPME/GC-MS	(Matysik, *et al.*, 2008)
Trimethylcyclohexanol						+																																			HS-SPME/GC-MS	(Fiedler, *et al.*, 2001)
1,10-Dimethyl-9-decalinol																																+									SPME/GC-MS	(Matysik, *et al.*, 2008)
1-Dodecanol																+																									HS-SPME/ GC-MS	(Martins, *et al.*, 2007, Martins, *et al.*, 2010)
Geosmin																														+				+							HS-SPME/GC-MS	(Nilsson, *et al.*, 1996)
Aromatics																																											
Benzenemethanol																						+										+									HS-SPME/GC-MS	(Fiedler, *et al.*, 2001)
2-Methylphenol																									+																	AE/GC-MS	(Fischer, *et al.*, 1999)
3-Methylphenol																																										(Fiedler, *et al.*, 2001)
2,6-Dimethylphenol						+																																			HS-SPME/GC-MS	(Fiedler, *et al.*, 2001)
2-Phenylethanol																																						+	+		+		SPME/GC-MS	(Osorio-Cadavid, *et al.*, 2008)
																	+																								SPME/GC-MS	(Matysik, *et al.*, 2008)
																																+								+	HS-SPME/GC-MS	(Fiedler, *et al.*, 2001)
																+																									HS-SPME/GC-MS	(Martins, *et al.*, 2007, Martins, *et al.*, 2010)
Aldehydes *Aliphatics*																																										
Acetaldehyde					+	+										+		+				+																			HS/SIFT-MS	(Scotter, *et al.*, 2005)
						+			+							+		+																					+		DHS/GC-MS	(Caileux, *et al.*, 1992)

Annexes

Metabolites	Microorganism																																										Method	Reference
	A														B	C	D	E	F	G	H		I	J															K		L	M	N		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14							1	2		1	2	3	4	5	6	7	8	9	10	11	12	1	2					
2-Butenal															+			+		+		+																					HS/SIFT-MS	(Scotter, *et al.*, 2005)
3-Methylbutanal		+						+																																			SPME/GC-MS	(Jelen & Grabarkiewicz-Szczesna, 2005)
Aromatics																																											
Furfural																																				+					+		SPME/GC-MS	(Osorio-Cadavid, *et al.*, 2008)
5-Methyl-furfural																																				+					+		SPME/GC-MS	(Osorio-Cadavid, *et al.*, 2008)
Benzaldehyde																																				+	+				+		SPME/GC-MS	(Osorio-Cadavid, *et al.*, 2008)
Esters ***Aliphatics***																																											
Ethyl acetate						+									+																									+	+	DHS/GC-MS	(Caileux, *et al.*, 1992)
																																				+	+						SPME/GC-MS	(Osorio-Cadavid, *et al.*, 2008)
Ethyl propanoate									+						+		+																									+	GC-MS	(Caileux, *et al.*, 1992)
Methyl isobutyrate																																		+										HS-SPME/GC-MS	(Fiedler, *et al.*, 2001)
2,3-Dimethyl-butanoic acid methyl ester																			+																									AE/ GC-MS	(Fischer, *et al.*, 1999)
Ethyl butanoate									+						+		+																											DHS/GC-MS	(Caileux, *et al.*, 1992)
																																		+								+		HS-SPME/GC-MS	(Fiedler, *et al.*, 2001)
2-Methylbutanoic acid methyl ester																			+																									AE/GC-MS	(Fischer, *et al.*, 1999)
Amyl acetate																																				+	+				+		SPME/GC-MS	(Osorio-Cadavid, *et al.*, 2008)
Ethyl tiglate									+																		+																SPME/GC-MS	(Matysik, *et al.*, 2008)
																																		+										HS-SPME/GC-MS	(Fiedler, *et al.*, 2001)
Isoamyl acetate																																				+	+						SPME/GC-MS	(Osorio-Cadavid, *et al.*, 2008)
																																		+										HS-SPME/GC-MS	(Fiedler, *et al.*, 2001)
															+		+																									+	DHS/GC-MS	(Caileux, *et al.*, 1992)
2-Methylbutanoic acid ethyl ester																												+																SPME/GC-MS	(Matysik, *et al.*, 2008)
																																		+								+		HS-SPME/GC-MS	(Fiedler, *et al.*, 2001)
3-Methylbutanoic acid ethyl ester																												+																SPME/GC-MS	(Matysik, *et al.*, 2008)
																																		+										HS-SPME/GC-MS	(Fiedler, *et al.*, 2001)
3-Methyl-2-butenoic acid ethyl ester									+																			+																SPME/GC-MS	(Matysik, *et al.*, 2008)
Diethyl succinate																																										+		SPME/GC-MS	(Osorio-Cadavid, *et al.*, 2008)
Ethyl hexanoate																																				+	+				+		SPME/GC-MS	(Osorio-Cadavid, *et al.*, 2008)
		+																																									AE/GC-MS	(Fischer, *et al.*, 1999)

Annexes

Metabolites	Microorganism																																				Method	Reference
	A														B	C	D	E	F	G	H		I	J													K		L	M	N	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14							1	2		1	2	3	4	5	6	7	8	9	10	11	12	1	2				
Hexyl ethanoate																																+								HS-SPME/GC-MS	(Fiedler, *et al.*, 2001)
																																				+					SPME/GC-MS	(Osorio-Cadavid, *et al.*, 2008)
Acetic acid ethylhexyl ester																																+								SPME/GC-MS	(Matysik, *et al.*, 2008)
Ethyl caprylate																																				+	+		+		SPME/GC-MS	(Osorio-Cadavid, *et al.*, 2008)
3-Octanol acetate																															+										HS-SPME/GC-MS	(Nilsson, *et al.*, 1996)
1-Octene-3-ol acetate																															+										HS-SPME/GC-MS	(Nilsson, *et al.*, 1996)
2-Methyl butyric acid isopentyl ester																																+								HS-SPME/GC-MS	(Fiedler, *et al.*, 2001)
Iso-amyl tiglate									+																	+														SPME/GC-MS	(Matysik, *et al.*, 2008)
3-Methylbutanoic acid i-pentyl ester									+																	+														SPME/GC-MS	(Matysik, *et al.*, 2008)
Ethyl nonanoate																																				+					SPME/GC-MS	(Osorio-Cadavid, *et al.*, 2008)
Ethyl caprate																																							+		SPME/GC-MS	(Osorio-Cadavid, *et al.*, 2008)
Isoamyl caprylate																																							+		SPME/GC-MS	(Osorio-Cadavid, *et al.*, 2008)
Ethyl palmitate	+								+																																SPME/GC-MS	(Jelen & Grabarkiewicz-Szczesna, 2005)
Ethyl linoleate	+								+																																SPME/GC-MS	(Jelen & Grabarkiewicz-Szczesna, 2005)
Aromatics																																										
Methyl benzoate						+																																		SPME/GC-MS	(Matysik, *et al.*, 2008)
2-Phenylethyl acetate																																				+	+		+		SPME/GC-MS	(Osorio-Cadavid, *et al.*, 2008)
Ethers ***Aliphatics***																																										
Butoxyethoxyethanol						+																																		SPME/GC-MS	(Matysik, *et al.*, 2008)
1-Methoxy-2-methylpropane																																+								HS-SPME/GC-MS	(Fiedler, *et al.*, 2001)
1-Methoxy-3-methylbutane																																+								HS-SPME/GC-MS	(Fiedler, *et al.*, 2001)
Aromatics																																										
Methoxybenzene			+											+																											AE/GC-MS	(Fischer, *et al.*, 1999)
1,2-Dimethoxybenzene														+																											AE/GC-MS	(Fischer, *et al.*, 1999)
1,3-Dimethoxybenzene														+												+															SPME/GC-MS	(Matysik, *et al.*, 2008)
3-Methylanisole																											+					+							+	HS-SPME/GC-MS	(Fiedler, *et al.*, 2001)
																										+					+									SPME/GC-MS	(Matysik, *et al.*, 2008)
																																				+					HS-SPME/GC-MS	(Nilsson, *et al.*, 1996)
																										+														AE/GC-MS	(Fischer, *et al.*, 1999)

Annexes

Metabolites	Microorganism																																					Method	Reference
	A															B	C	D	E	F	G	H		I	J													K		L	M	N	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14								1	2		1	2	3	4	5	6	7	8	9	10	11	12	1	2				
1,4-Dimethoxy-2-methylbenzene																									+																SPME/GC-MS	(Matysik, *et al.*, 2008)
2,5-Dimethoxytoluene									+																	+							+								HS-SPME/GC-MS	(Fiedler, *et al.*, 2001)
Dimethylanisole																					+																				HS-SPME/GC-MS	(Fiedler, *et al.*, 2001)
4-Ethylanisole																																	+								HS-SPME/GC-MS	(Fiedler, *et al.*, 2001)
1,2,3-trimethoxybenzene							+					+																													SPME/GC-MS	(Jelen & Grabarkiewicz-Szczesna, 2005)
Furan – type compounds																																										
2-Methylfuran																																	+								HS-SPME/GC-MS	(Fiedler, *et al.*, 2001)
3-Methylfuran									+																																SPME/GC-MS	(Matysik, *et al.*, 2008)
										+											+												+								HS-SPME/GC-MS	(Fiedler, *et al.*, 2001)
Furaneol																													+												AE/GC-MS	(Fischer, *et al.*, 1999)
2,5-Dimethylfuran																													+	+											AE/GC-MS	(Fischer, *et al.*, 1999)
																					+												+								HS-SPME/GC-MS	(Fiedler, *et al.*, 2001)
2-Ethylfuran																													+												AE/GC-MS	(Fischer, *et al.*, 1999)
2-Acetyl-5-methylfuran																									+																AE/GC-MS	(Fischer, *et al.*, 1999)
2-Ethyl-5-methyl-furan																													+												AE/GC-MS	(Fischer, *et al.*, 1999)
Isopropylfuran																													+												AE/GC-MS	(Fischer, *et al.*, 1999)
2,3,5-Trimethylfuran			+																						+				+	+											AE/GC-MS	(Fischer, *et al.*, 1999)
Hydrocarbons ***Aliphatics***																																										
Isoprene						+				+																														+	HS-SPME/GC-MS	(Fiedler, *et al.*, 2001)
2,4-Hexadiene						+																																			HS-SPME/GC-MS	(Fiedler, *et al.*, 2001)
2-Methyl-1,3-pentadiene						+																																			HS-SPME/GC-MS	(Fiedler, *et al.*, 2001)
Heptane						+								+																										+	HS-SPME/GC-MS	(Fiedler, *et al.*, 2001)
	+				+		+	+	+	+		+	+																												SPME/GC-MS	(Jelen & Grabarkiewicz-Szczesna, 2005)
1,3,5-Heptatriene										+																														+	HS-SPME/GC-MS	(Fiedler, *et al.*, 2001)
1-Heptene																									+																SPME/GC-MS	(Matysik, *et al.*, 2008)
2-Methyl-2,4-hexadiene																									+																AE/GC-MS	(Fischer, *et al.*, 1999)
Cyclooctatriene										+																															HS-SPME/GC-MS	(Fiedler, *et al.*, 2001)

Annexes

Metabolites	Microorganism																																										Method	Reference
	A															B	C	D	E	F	G	H		I	J															K		L	M	N		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14							1	2		1	2	3	4	5	6	7	8	9	10	11	12		1	2					
1,3,5-Cyclooctatriene																																+												AE/GC-MS	(Fischer, *et al.*, 1999)
Cyclooctene																				+									+														AE/GC-MS	(Fischer, *et al.*, 1999)
3-Methyl-1-heptene			+																							+																		AE/GC-MS	(Fischer, *et al.*, 1999)
1,3-Octadiene						+				+			+	+												+						+											+	HS-SPME/GC-MS	(Fiedler, *et al.*, 2001)
														+			+									+						+												SPME/GC-MS	(Matysik, *et al.*, 2008)
																																			+	+									HS-SPME/GC-MS	(Nilsson, *et al.*, 1996)
1,3,6-Octadiene			+																																										AE/GC-MS	(Fischer, *et al.*, 1999)
2,4,6-Octatriene														+														+																	HS-SPME/GC-MS	(Fiedler, *et al.*, 2001)
1-Octene																	+									+																			SPME/GC-MS	(Matysik, *et al.*, 2008)
								+																																					SPME/GC-MS	(Jelen & Grabarkiewicz-Szczesna, 2005)
1,3-*Trans*-5-*cis*-octatriene																																				+	+								HS-SPME/GC-MS	(Nilsson, *et al.*, 1996)
2,2,3,3-Tetramethylbutane	+	+		+	+		+		+		+	+	+	+																														SPME/GC-MS	(Jelen & Grabarkiewicz-Szczesna, 2005)
Nonane														+																															HS-SPME/GC-MS	(Fiedler, *et al.*, 2001)
1,3-Nonadiene																										+																			HS-SPME/GC-MS	(Fiedler, *et al.*, 2001)
									+								+									+																		SPME/GC-MS	(Matysik, *et al.*, 2008)
1-Nonene																										+																			SPME/GC-MS	(Matysik, *et al.*, 2008)
1,2,3-Trimethylcyclohexane			+																																										AE/GC-MS	(Fischer, *et al.*, 1999)
Decane														+								+																							HS-SPME/GC-MS	(Fiedler, *et al.*, 2001)
2,6-Dimethyl-2,4,6-octatriene																																												+	HS-SPME/GC-MS	(Fiedler, *et al.*, 2001)
Undecane						+								+																															HS-SPME/GC-MS	(Fiedler, *et al.*, 2001)
Dodecane																													+																AE/GC-MS	(Fischer, *et al.*, 1999)
Tetradecene																	+									+																			SPME/GC-MS	(Matysik, *et al.*, 2008)
Pentadecene									+								+									+																			SPME/GC-MS	(Matysik, *et al.*, 2008)
Hexadecane																										+																			SPME/GC-MS	(Matysik, *et al.*, 2008)
Aromatics																																														
Toluene										+				+								+																						+	HS-SPME/GC-MS	(Fiedler, *et al.*, 2001)
Ethylbenzene														+																															HS-SPME/GC-MS	(Fiedler, *et al.*, 2001)
Xylene	+	+		+	+		+	+			+	+	+	+																														SPME/GC-MS	(Jelen & Grabarkiewicz-Szczesna, 2005)
														+							+																						+	HS-SPME/GC-MS	(Fiedler, *et al.*, 2001)
Styrene																							+	+				+	+			+	+									+	AE/GC-MS	(Fischer, *et al.*, 1999)
																																+										+	HS-SPME/GC-MS	(Fiedler, *et al.*, 2001)
1-Ethyl-2-methylbenzene			+																																									AE/GC-MS	(Fischer, *et al.*, 1999)
Trimethylbenzene																					+																							HS-SPME/GC-MS	(Fiedler, *et al.*, 2001)

Annexes

Metabolites	Microorganism																																					Method	Reference
	A														B	C	D	E	F	G	H		I	J													K		L	M	N	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14							1	2		1	2	3	4	5	6	7	8	9	10	11	12	1	2					
Trimethylnaphthalene														+																										HS-SPME/GC-MS	(Fiedler, *et al.*, 2001)
Ketones ***Aliphatics***																																									
2-Propanone					+	+									+						+																		HS/SIFT-MS	(Scotter, *et al.*, 2005)
						+			+					+																	+								+	HS-SPME/GC-MS	(Fiedler, *et al.*, 2001)
2-Butanone														+																	+									HS-SPME/GC-MS	(Fiedler, *et al.*, 2001)
Cyclopentanone						+																																		HS-SPME/GC-MS	(Fiedler, *et al.*, 2001)
2-Pentanone						+			+					+		+								+							+									SPME/GC-MS	(Matysik, *et al.*, 2008)
						+								+																	+								+	HS-SPME/GC-MS	(Fiedler, *et al.*, 2001)
2,4-Pentandione						+																																		SPME/GC-MS	(Matysik, *et al.*, 2008)
2-Hexanone						+								+																	+								+	HS-SPME/GC-MS	(Fiedler, *et al.*, 2001)
3-Hexanone																								+																SPME/GC-MS	(Fischer, *et al.*, 1999)
						+								+																	+								+	HS-SPME/GC-MS	(Fiedler, *et al.*, 2001)
3-Methyl-1,3-pentandione						+																																		SPME/GC-MS	(Matysik, *et al.*, 2008)
3-Cyclehepten-1-one			+																																					AE/GC-MS	(Fischer, *et al.*, 1999)
2-Heptanone						+			+					+		+								+							+									SPME/GC-MS	(Matysik, *et al.*, 2008)
						+								+											+						+								+	HS-SPME/GC-MS	(Fiedler, *et al.*, 2001)
4-Heptanone						+																																	+	HS-SPME/GC-MS	(Fiedler, *et al.*, 2001)
4-Methyl-3-hexanone																																+								HS-SPME/GC-MS	(Fiedler, *et al.*, 2001)
																								+																AE/GC-MS	(Fischer, *et al.*, 1999)
Bicyclooctan-2-one																										+														AE/GC-MS	(Fischer, *et al.*, 1999)
Bicyclo-(3,2,1)-octan-2-one																										+														AE/GC-MS	(Fischer, *et al.*, 1999)
2,5-Methyl-3-heptanone																																+								HS-SPME/GC-MS	(Fiedler, *et al.*, 2001)
4-Methyl-6-hepten-3-one														+																										HS-SPME/GC-MS	(Fiedler, *et al.*, 2001)
6-Methyl-2-heptanone														+																										AE/GC-MS	(Fischer, *et al.*, 1999)
2-Octanone						+								+													+					+								+	HS-SPME/GC-MS	(Fiedler, *et al.*, 2001)
3-Octanone			+																																					AE/GC-MS	(Fischer, *et al.*, 1999)
																														+	+			+					HS-SPME/GC-MS	(Nilsson, *et al.*, 1996)
						+			+					+		+								+							+									SPME/GC-MS	(Matysik, *et al.*, 2008)
						+			+					+																	+								+	HS-SPME/GC-MS	(Fiedler, *et al.*, 2001)
	+	+		+	+		+	+	+		+	+	+	+																										SPME/GC-MS	(Jelen & Grabarkiewicz-Szczesna, 2005)
2-Nonanone						+								+										+																SPME/GC-MS	(Matysik, *et al.*, 2008)
5-Ethyl-4-methyl-3-heptanone														+																										SPME/GC-MS	(Matysik, *et al.*, 2008)

Annexes

Metabolites	Microorganism																																						Method	Reference
	A														B	C	D	E	F	G	H		I	J													K		L	M	N	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14							1	2		1	2	3	4	5	6	7	8	9	10	11	12	1	2				
Aromatics																																								
Acetophenone						+																																	HS-SPME/GC-MS	(Fiedler, *et al.*, 2001)
1-(3-Methylphenyl)-ethanone														+																									AE/GC-MS	(Fischer, *et al.*, 1999)
2,6-di-*tert*-butyl-*p*-benzoquinone														+																									SPME/GC-MS	(Matysik, *et al.*, 2008)
N- compounds																																								
Pyrazine														+																+									HS-SPME/GC-MS	(Fiedler, *et al.*, 2001)
Methylpyrazine						+								+						+						+				+									HS-SPME/GC-MS	(Fiedler, *et al.*, 2001)
Pyridine																+																								SPME/GC-MS	(Matysik, *et al.*, 2008)
						+																																+		HS-SPME/GC-MS	(Fiedler, *et al.*, 2001)
2-Isopropyl-3-methoxy-pyrazine																														+									HS-SPME/GC-MS	(Fiedler, *et al.*, 2001)
S - compounds																																								
Methanethiol																				+		+																		HS/SIFT-MS	(Scotter, *et al.*, 2005)
																														+										SPME/GC-MS	(Matysik, *et al.*, 2008)
Dimethyl disulfide																					+									+										HS-SPME/GC-MS	(Fiedler, *et al.*, 2001)
																											+													AE/GC-MS	(Fischer, *et al.*, 1999)
Dimethyl sulfide																														+										HS-SPME/GC-MS	(Fiedler, *et al.*, 2001)
Terpenes																																								
Monoterpenes																																								
α-Pinene						+																																	AE/GC-MS	(Fischer, *et al.*, 1999)
																																					+		HS-SPME/GC-MS	(Nilsson, *et al.*, 1996)
α-Phellandrene																							+																	AE/GC-MS	(Fischer, *et al.*, 1999)
α-Terpineol																														+										SPME/GC-MS	(Matysik, *et al.*, 2008)
α-Terpinene																							+				+													AE/GC-MS	(Fiedler, *et al.*, 2001)
α-Terpinolene																				+																				AE/GC-MS	(Fischer, *et al.*, 1999)
β-fenchyl alcohol																				+																				AE/GC-MS	(Fischer, *et al.*, 1999)
β-Phellandrene																							+																	AE/GC-MS	(Fischer, *et al.*, 1999)
β-Myrcene																					+						+													HS-SPME/GC-MS	(Fiedler, *et al.*, 2001)
																																						+		HS-SPME/GC-MS	(Nilsson, *et al.*, 1996)
β-Pinene																																						+		HS-SPME/GC-MS	(Nilsson, *et al.*, 1996)
Borneol																																						+		HS-SPME/GC-MS	(Nilsson, *et al.*, 1996)
Bornylene																																				+				HS-SPME/GC-MS	(Nilsson, *et al.*, 1996)

Annexes

Metabolites	Microorganism																																								Method	Reference
	A														B	C	D	E	F	G	H		I	J															K		L	M	N		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14							1	2		1	2	3	4	5	6	7	8	9	10	11	12	1	2					
1,8-Cineole																																		+	+						HS-SPME/GC-MS	(Nilsson, *et al.*, 1996)
Camphene						+																																			AE/GC-MS	(Fischer, *et al.*, 1999)
																																			+						HS-SPME/GC-MS	(Nilsson, *et al.*, 1996)
Camphor																																			+						HS-SPME/GC-MS	(Nilsson, *et al.*, 1996)
δ-4-Carene																							+																		AE/GC-MS	(Fischer, *et al.*, 1999)
δ-2-Dodecanol																													+												AE/GC-MS	(Fischer, *et al.*, 1999)
Eucalyptol																											+														HS-SPME/GC-MS	(Fiedler, *et al.*, 2001)
y-Terpinene																																			+						HS-SPME/GC-MS	(Nilsson, *et al.*, 1996)
Limonene						+								+					+				+				+	+				+									AE/GC-MS	(Fischer, *et al.*, 1999)
						+								+						+						+									+					+	HS-SPME/GC-MS	(Fiedler, *et al.*, 2001)
																																			+						HS-SPME/GC-MS	(Nilsson, *et al.*, 1996)
	+	+		+	+		+	+	+		+	+	+	+																											SPME/GC-MS	(Jelen & Grabarkiewicz-Szczesna, 2005)
Linalol																														+					+						HS-SPME/GC-MS	(Nilsson, *et al.*, 1996)
Myrcene														+									+				+														AE/GC-MS	(Fischer, *et al.*, 1999)
Myrtenol										+																															HS-SPME/GC-MS	(Fiedler, *et al.*, 2001)
Sabinene																																			+						HS-SPME/GC-MS	(Nilsson, *et al.*, 1996)
Terpinolene																																			+						HS-SPME/GC-MS	(Nilsson, *et al.*, 1996)
Terpinen-4-ol														+													+														HS-SPME/GC-MS	(Fiedler, *et al.*, 2001)
2-Methylenebornane																												+	+													AE/GC-MS	(Fischer, *et al.*, 1999)
2-Methyl-2-bornene																													+													AE/GC-MS	(Fischer, *et al.*, 1999)
2-Methylisoborneol																															+				+							HS-SPME/GC-MS	(Nilsson, *et al.*, 1996)
Butyl caprylate																																							+			SPME/GC-MS	(Osorio-Cadavid, *et al.*, 2008)
Sesquiterpenes																																											
Acoradiene																															+				+							HS-SPME/GC-MS	(Nilsson, *et al.*, 1996)
Aromadendrene																																+										AE/GC-MS	(Fischer, *et al.*, 1999)
α-Bisabolene						+																					+															HS-SPME/GC-MS	(Fiedler, *et al.*, 2001)
α-Chamigrene																																		+								HS-SPME/GC-MS	(Nilsson, *et al.*, 1996)
α-Copaene																																			+							HS-SPME/GC-MS	(Nilsson, *et al.*, 1996)

Annexes

Metabolites	Microorganism																																							Method	Reference
	A														B	C	D	E	F	G	H		I	J															K		L	M	N	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14							1	2		1	2	3	4	5	6	7	8	9	10	11	12	1	2					
α-Cubebene																					+														+						+	HS-SPME/GC-MS	(Fiedler, *et al.*, 2001)
																																		+							HS-SPME/GC-MS	(Nilsson, *et al.*, 1996)
									+																																	SPME/GC-MS	(Jelen & Grabarkiewicz-Szczesna, 2005)
α-Farnesene						+																																				AE/GC-MS	(Fischer, *et al.*, 1999)
						+																																				HS-SPME/GC-MS	(Fiedler, *et al.*, 2001)
α-Longipinene																							+																			AE/GC-MS	(Fischer, *et al.*, 1999)
α-Muurolene														+																			+									AE/GC-MS	(Fischer, *et al.*, 1999)
cis-α-bergamotene																													+													HS-SPME/GC-MS	(Nilsson, *et al.*, 1996)
trans-α-bergamotene																													+						+							HS-SPME/GC-MS	(Nilsson, *et al.*, 1996)
Bicycloelemene																															+											AE/GC-MS	(Fischer, *et al.*, 1999)
β-Bisabolene																																			+							HS-SPME/GC-MS	(Nilsson, *et al.*, 1996)
β-Caryophyllene																																			+							HS-SPME/GC-MS	(Nilsson, *et al.*, 1996)
																											+															AE/GC-MS	(Fischer, *et al.*, 1999)
β-Chamigrene																													+						+							HS-SPME/GC-MS	(Nilsson, *et al.*, 1996)
β-Elemene																												+															AE/GC-MS	(Fischer, *et al.*, 1999)
β-Farnesene						+																					+				+											HS-SPME/GC-MS	(Fiedler, *et al.*, 2001)
β-Himachalene																													+						+							HS-SPME/GC-MS	(Nilsson, *et al.*, 1996)
														+													+															HS-SPME/GC-MS	(Fiedler, *et al.*, 2001)
β-Maaliene																																			+							HS-SPME/GC-MS	(Nilsson, *et al.*, 1996)
Trans-β-farnesene						+																																				AE/GC-MS	(Fischer, *et al.*, 1999)
Chamigrene														+																											+	HS-SPME/GC-MS	(Fiedler, *et al.*, 2001)
E,E-farnesol															+																												HS-SPME/GC-MS	(Martins, *et al.*, 2007, Martins, *et al.*, 2010)
E-nerolidol															+																												HS-SPME/GC-MS	(Martins, *et al.*, 2007, Martins, *et al.*, 2010)
Elemene																												+															HS-SPME/GC-MS	(Fiedler, *et al.*, 2001)
Elemol																															+	+										AE/GC-MS	(Fischer, *et al.*, 1999)
Germacrene A																														+													AE/GC-MS	(Fischer, *et al.*, 1999)
Germacrene B																							+								+												AE/GC-MS	(Fischer, *et al.*, 1999)
Longifolene																													+													HS-SPME/GC-MS	(Nilsson, *et al.*, 1996)
Longipinene														+																											+	HS-SPME/GC-MS	(Fiedler, *et al.*, 2001)
Pachoulene																																									+	HS-SPME/GC-MS	(Fiedler, *et al.*, 2001)
χ-Cadinene																							+							+												AE/GC-MS	(Fischer, *et al.*, 1999)

Annexes

Metabolites	Microorganism																																						Method	Reference
	A														B	C	D	E	F	G	H		I	J														K		L	M	N		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14							1	2		1	2	3	4	5	6	7	8	9	10	11	12	1	2					
χ-Curcumene														+										+																AE/GC-MS	(Fischer, *et al.*, 1999)
Thujopsene																								+																HS-SPME/GC-MS	(Nilsson, *et al.*, 1996)
Widdrol																								+																HS-SPME/GC-MS	(Nilsson, *et al.*, 1996)

A1- *Aspergillus alliaceus*; **A2**- *Aspergillus amstelodami*; **A3**- *Aspergillus candidus;* **A4**- *Aspergillus fisherii*; **A5**- *Aspergillus flavus*; **A6**- *Aspergillus fumigatus*; **A7**- *Aspergillus glaucus*; **A8**- *Aspergillus melleus*; **A9**- *Aspergillus niger*; **A10**- *Aspergillus ochraceus*; **A11**- *Aspergillus ostianus*; **A12**- *Aspergillus repens*; **A13**- *Aspergillus terreus*; **A14**- *Aspergillus versicolor*; **B**- *Candida albicans*; **C**- *Cladosporium cladosporoides*; **D**- *Clavispora lusitaniae*; **E**- *Cryptococcus neoformans*; **F**- *Emericella nidulans*; **G**- *Fusarium solani*; **H1**- *Mucor* sp.; **H2**- *Mucor racemosus*; **I**- *Paecilomyces variotti*; **J1**- *Penicillium brevicompactum*; **J2**- *Penicillium chrysogenum*; **J3**- *Penicillium claviforme*; **J4**- *Penicillium clavigerum*; **J5**- *Penicillium crustosum*; **J6**= *Penicillium cyclopium*; **J7**- *Penicillium decumbens*; **J8**- *Penicillium discolor*; **J9**- *Penicillium expansum*; **J10**- *Penicillium glabrum*; **J11**- *Penicillium hirsutum var. venetum*; **J12**- *Penicillium vulpinum*; **K1**- *Pichia fermentans*; **K2**- *Pichia kluyveri var. kluyveri*; **L**-*Rhizopus oryzae*; **M**- *Saccharomyces cerevisiae*; **N**- *Trichoderma harzianum*;

Methods:
AE– Adsorvent extraction
DHS – Dynamic headspace
DS – Direct sampling
GC-MS– Gas chromatography-mass spectrometry
HS –Headspace
SIFT-MS – Selected ion flow tube-mass spectrometry
SPME– Solid phase microextraction

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Table S2 – Chromatographic data of 504 volatile compounds identified from *A. niger* cultures in YGC medium (solid and liquid) by using HS-SPME/GC×GC-ToFMS.

Peak	$^{1}t_R$ [a] (s)	$^{2}t_R$ [a] (s)	Compound	Formula	CAS	R.I. $_{Calc}$ [b]	R.I. $_{Lit}$ [c]		R.I. Reference
							GC×GC	GC-MS	
			Acids						
1	185	0.550	2-Methylpropanoic acid	$C_4H_8O_2$	79-31-2	791	-	775	(Kotseridis & Baumes, 2000)
2	200	1.860	Butanoic acid	$C_4H_8O_2$	107-92-6	814	-	821	(Schnermann & Schieberle, 1997)
3	240	1.130	3-Methylbutanoic acid	$C_5H_{10}O_2$	503-74-2	860	-	864	(Figuérédo, *et al.*, 2006)
4	255	0.410	2-Methylbutanoic acid	$C_5H_{10}O_2$	116-53-0	877	-	873	(Schnermann & Schieberle, 1997)
5	485	3.370	2-Ethylhexanoic acid	$C_8H_{16}O_2$	149-57-5	1132	-	1129	(Adams, 1995)
6	535	3.680	Octanoic acid	$C_8H_{16}O_2$	124-07-2	1188	-	1182	(Wu, *et al.*, 2007)
7	615	3.200	Nonanoic acid	$C_9H_{18}O_2$	112-05-0	1280	-	1280	(Adams, 1995)
8	695	2.890	Decanoic acid	$C_{10}H_{20}O_2$	334-48-5	1379	-	1380	(Pino, *et al.*, 2005)
9	1020	2.650	Tetradecanoic acid	$C_{14}H_{28}O_2$	544-63-8	1774	-	1780	(Pino, *et al.*, 2005)
10	1105	2.590	Pentadecanoic acid	$C_{15}H_{30}O_2$	1002-84-2	1868	-	1878	(Pino, *et al.*, 2005)
			Alcohols						
			Aliphatics						
11	85	0.700	1-Propanol	C_3H_8O	71-23-8	580	-	574	(Rembold, *et al.*, 1989)
12	95	0.640	2-Butanol	$C_4H_{10}O$	78-92-2	601	603	-	(Xu, *et al.*, 2003)
13	100	0.800	2-Methyl-1-propanol	$C_4H_{10}O$	78-83-1	612	615	-	(Rocha, *et al.*, 2013)
14	115	0.910	1-Butanol	$C_4H_{10}O$	71-36-3	644	655	-	(Xu, *et al.*, 2003)
15	130	0.740	3-Pentanol	$C_5H_{12}O$	584-02-1	675	-	710	(Pino, *et al.*, 2005)
16	130	0.770	2-Pentanol	$C_5H_{12}O$	6032-29-7	675	-	685	(Guichard & Souty, 1988)
17	145	1.260	3-Methyl-3-buten-1-ol	$C_5H_{10}O$	763-32-6	708	-	716	(Rodriguez-Burruezo, *et al.*, 2004)
18	150	1.160	3-Methyl-1-butanol	$C_5H_{12}O$	123-51-3	718	706	-	(Rocha, *et al.*, 2013)
19	165	0.800	4-Methyl-2-pentanol	$C_6H_{14}O$	108-11-2	749	-	758	(Engel & Ratel, 2007)
20	170	0.770	2-Methyl-3-pentanol	$C_6H_{14}O$	565-67-3	760	-	774	(Turchimi, *et al.*, 2004)
21	170	1.120	1-Pentanol	$C_5H_{12}O$	71-41-0	760	776	-	(Salvador, *et al.*, 2013)
22	180	1.490	3-Methyl-2-buten-1-ol	$C_5H_{10}O$	556-82-1	782	-	778	(Adams, 1995)
23	185	0.860	3-Methyl-2-pentanol	$C_6H_{14}O$	565-60-6	791	-	-	-
24	190	0.820	3-Hexanol	$C_6H_{14}O$	623-37-0	801	-	806	(Boylston & Viniyard, 1998)
25	225	1.130	4-Methyl-1-pentanol	$C_6H_{14}O$	626-89-1	843	-	846	(Leffingwell & Alford, 2005)
26	245	1.400	4-Methyl-3-penten-1-ol	$C_6H_{12}O$	763-89-3	866	-	-	-
27	255	1.140	1-Hexanol	$C_6H_{14}O$	111-27-3	878	877	-	(Rocha, *et al.*, 2013)
28	255	1.480	4-Hexen-1-ol	$C_6H_{12}O$	928-92-7	878	-	883	(Zhao, *et al.*, 2008)
29	260	1.100	1-Hepten-3-ol	$C_7H_{14}O$	4938-52-7	884	-	881	(Berdague, *et al.*, 2007)
30	270	0.820	4-Heptanol	$C_7H_{16}O$	589-55-9	895	-	879	(Mahmood, *et al.*, 2004)
31	275	0.840	3-Heptanol	$C_7H_{16}O$	589-82-2	901	892	-	(Xu, *et al.*, 2003)
32	280	0.890	2-Heptanol	$C_7H_{16}O$	543-49-7	906	-	904	(Rembold, *et al.*, 1989)
33	310	1.140	2-Hepten-1-ol (isomer)	$C_7H_{14}O$	55454-22-3	938	-	-	-
34	315	0.840	Butoxypropanol	$C_7H_{16}O_2$	5131-66-8	943	-	947	(Forero, *et al.*, 2009)
35	330	1.150	5-Methyl-1-hepten-4-ol	$C_8H_{16}O$	99328-46-8	959	-	-	-

36	335	0.870	2-Octanol	$C_8H_{18}O$	123-96-6	964	-	962	(Nogueira, *et al.*, 2001)
37	345	1.100	1-Heptanol	$C_7H_{16}O$	111-70-6	975	974	-	(Salvador, *et al.*, 2013)
38	345	1.380	2-Hepten-1-ol	$C_7H_{14}O$	33467-76-4	975	-	970	(Rembold, *et al.*, 1989)
39	350	1.050	1-Octen-3-ol	$C_8H_{16}O$	3391-86-4	980	992	-	(Silva, *et al.*, 2010)
40	350	1.210	Octa-1,5-dien-3-ol	$C_8H_{14}O$	50306-18-8	980	-	-	-
41	355	0.930	5-Octen-3-ol	$C_8H_{16}O$		985	-	-	-
42	365	1.010	6-Methyl-1-heptanol	$C_8H_{18}O$	1653-40-3	996	-	-	-
43	365	1.030	6-Methyl-5-hepten-2-ol	$C_8H_{16}O$	1569-60-4	996	-	995	(Zhao, *et al.*, 2008)
44	365	1.270	3-Octanol	$C_8H_{18}O$	589-98-0	996	-	996	(Zhao, *et al.*, 2006)
45	390	1.100	3-Ethyl-4-methyl-1-pentanol	$C_8H_{18}O$	100431-87-6	1023	-	-	-
46	395	0.990	2-Ethyl- 1-hexanol	$C_8H_{18}O$	104-76-7	1029	1038	-	(Silva, *et al.*, 2010)
47	405	1.040	4-Methyl-1-heptanol	$C_8H_{18}O$	817-91-4	1040	-	-	-
48	415	1.030	5-Methyl-1-heptanol	$C_8H_{18}O$	7212-53-5	1051	-	-	-
49	435	0.790	2,6-Dimethyl-7-octen-2-ol	$C_{10}H_{20}O$	18479-58-8	1073	-	1075	(Diaz & Kite, 2002)
50	435	1.280	2-Octen-1-ol	$C_8H_{16}O$	18409-17-1	1074	-	1066	(Pino, *et al.*, 2004)
51	440	0.740	1-Nonen-3-ol	$C_9H_{18}O$	21964-44-3	1079	-	1079	(Berdague, *et al.*, 2007)
52	440	1.030	1-Octanol	$C_9H_{18}O_2$	111-87-5	1079	1079	-	(Silva, *et al.*, 2010)
53	445	0.700	2,6-Dimethyl-1,7-octadien-3-ol	$C_{10}H_{18}O$	22460-59-9	1084	1095	-	(Rocha, *et al.*, 2007)
54	460	0.700	2,6-Dimethyl-2-octanol	$C_{10}H_{22}O$	18479-57-7	1101	-	-	-
55	460	0.840	2-Nonanol	$C_9H_{20}O$	628-99-9	1101	-	1098	(Adams, 1995)
56	465	0.990	7-Octen-2-ol	$C_8H_{16}O$	39546-75-3	1107	-	-	-
57	480	1.030	2-Nonen-1-ol (isomer)	$C_9H_{18}O$	31502-14-4	1123	1074	-	(Caldeira, *et al.*, 2011)
58	490	1.010	1-Octen-4-ol	$C_8H_{16}O$	40575-42-6	1135	-	-	-
59	505	1.040	2-Nonen-1-ol (isomer)	$C_9H_{18}O$	-	1151	-	-	-
60	525	0.970	1-Nonanol	$C_9H_{20}O$	143-08-8	1173	1179	-	(Silva, *et al.*, 2010)
61	530	1.110	2-Nonen-1-ol (isomer)	$C_9H_{18}O$	22104-79-6	1179	-	-	-
62	545	0.760	3-Decanol	$C_{10}H_{22}O$	1565-81-7	1195	-	1188	(Adams, 1995)
63	545	1.360	2-(2-Butoxyethoxy)-ethanol	$C_8H_{18}O_3$	112-34-5	1196	1192	-	(Dallüge, *et al.*, 2002)
64	565	0.860	2-Propyl-1-heptanol	$C_{10}H_{22}O$	10042-59-8	1219	-	-	-
65	615	0.620	2-Decen-1-ol	$C_{10}H_{20}O$	18409-18-2	1277	1281	-	(Eyres, *et al.*, 2005)
66	615	0.910	1-Decanol	$C_{10}H_{22}O$	112-30-1	1278	1281	-	(Silva, *et al.*, 2010)
67	625	0.730	4-Undecanol	$C_{11}H_{24}O$	4272-06-4	1289	-	1281	(Mahmood, *et al.*, 2004)
68	630	0.700	2,4-Undecadien-1-ol	$C_{11}H_{20}O$	77657-78-4	1295	-	-	-
69	635	0.770	2-Undecanol	$C_{11}H_{24}O$	1653-30-1	1301	-	1303	(Setzer, *et al.*, 2005)
70	645	0.830	2-Butyl-1-octanol	$C_{12}H_{26}O$	3913-02-8	1314	-	-	-
71	725	0.860	2-Dodecanol	$C_{12}H_{26}O$	10203-28-8	1414	1413	-	(Silva, *et al.*, 2010)
72	760	0.960	6,10-Dimethyl-5,9-undecadien-2-ol	$C_{13}H_{24}O$	53837-34-6	1458	-	1459	(Dickschat, *et al.*, 2005)
73	775	0.940	1-Dodecanol	$C_{12}H_{26}O$	112-53-8	1476	1480	-	(Silva, *et al.*, 2010)
74	950	0.970	1-Tetradecanol	$C_{14}H_{30}O$	112-72-1	1684	1686	-	(Silva, *et al.*, 2010)
			Aromatics						
75	410	4.130	Benzyl alcohol	C_7H_8O	100-51-6	1049	1044	-	(Robinson, *et al.*, 2011)
76	430	2.770	Methylbenzenemethanol	$C_8H_{10}O$	1445-91-6	1070	-	-	-
77	450	1.990	2-Phenylisopropanol	$C_9H_{12}O$	617-94-7	1091	1080	-	(Kallio, *et al.*, 2006)
78	455	1.070	4-Ethyl-1,3-benzenediol	$C_8H_{10}O_2$	2896-60-8	1096	-	-	-
79	475	3.030	2-Phenylethanol	$C_8H_{10}O$	60-12-8	1120	1107	-	(Weldegergis, *et al.*, 2011)

80	495	1.940	Methylbenzeneethanol	$C_9H_{12}O$	698-87-3	1141	-	-	-
81	555	2.590	2,4,6-Trimethylphenol	$C_9H_{12}O$	527-60-6	1209	-	1203	(Song, *et al.*, 2003)
82	680	2.700	2-(1,1-Dimethylethyl)-4-methylphenol	$C_{11}H_{16}O$	2409-55-4	1360	-	1387	(Aaslyng, *et al.*, 1998)
83	805	2.060	2,4-bis(1,1-dimethylethyl)-phenol	$C_{14}H_{22}O$	96-76-4	1514	-	1513	(Zhao, *et al.*, 2006)
84	810	0.780	Butyl hydroxy toluene	$C_{15}H_{24}O$	128-37-0	1519	-	1518	(Zhao, *et al.*, 2006)
85	890	3.240	(1,1,3,3-tetramethylbutyl)-phenol	$C_{14}H_{22}O$	27193-28-8	1616	-	-	-
			Cyclics						
86	165	1.470	Cyclopropaneethanol	$C_5H_{10}O$	2566-44-1	750	-	-	-
87	545	1.150	3-Methylcyclohexanol	$C_7H_{14}O$	591-23-1	1196	-	-	-
			Aldehydes						
			Aliphatics						
88	70	0.340	Acetaldehyde (*m/z* 44, 43, 42, 41)	C_2H_4O	75-07-0	548	-	500	(Qian & Reineccius, 2003)
89	110	0.460	3-Methylbutanal	$C_5H_{10}O$	590-86-3	633	628	-	(Loureiro, *et al.*, 2014)
90	110	0.650	2-Butenal	C_4H_6O	4170-30-3	633	657	-	(Dallüge, *et al.*, 2002)
91	115	0.440	2-Methylbutanal	$C_5H_{10}O$	96-17-3	643	635	-	(Loureiro, *et al.*, 2014)
92	120	0.490	*m/z* 55, 84, 39, 56	C_5H_8O	-	654	-	-	-
93	125	0.500	Pentanal	$C_5H_{10}O$	110-62-3	664	691	-	(Xu, *et al.*, 2003)
94	155	0.670	2-Methyl-2-butenal	C_5H_8O	497-03-0	728	-	739	(Bruna, *et al.*, 2001)
95	190	0.590	Hexanal	$C_6H_{12}O$	66-25-1	801	800	-	(Rocha, *et al.*, 2012)
96	195	0.710	3-Hexenal	$C_6H_{10}O$	6789-80-6	807	-	801	(Ruther, 2000)
97	275	0.620	Heptanal	$C_7H_{14}O$	111-71-7	901	903	-	(Rocha, *et al.*, 2012)
98	325	0.560	2-Ethylhexanal	$C_8H_{16}O$	123-05-7	953	955	-	(Xu, *et al.*, 2003)
99	330	0.790	2-Heptenal	$C_7H_{12}O$	18829-55-5	959	956	-	(Xu, *et al.*, 2003)
100	360	0.680	5-Methyl-2-heptenal	$C_8H_{14}O$	94705-03-0	990	-	-	-
101	370	0.640	Octanal	$C_8H_{16}O$	124-13-0	1001	1004	-	(Rocha, *et al.*, 2012)
102	415	0.740	2-Octenal (isomer)	$C_8H_{14}O$	2363-89-5	1051	1056	-	(Kallio, *et al.*, 2006)
103	420	0.650	2,6-Dimethyl-5-heptenal	$C_9H_{16}O$	106-72-9	1056	-	1060	(Avato, *et al.*, 2004)
104	425	0.780	2-Octenal (isomer)	$C_8H_{14}O$	2548-87-0	1062	1056	-	(Salvador, *et al.*, 2013)
105	425	0.910	2,6-Octadienal	$C_8H_{12}O$	76917-23-2	1062	-	-	-
106	430	0.640	2-Methylenehexanal	$C_7H_{12}O$	1070-66-2	1067	-	-	-
107	465	0.630	Nonanal	$C_9H_{18}O$	124-19-6	1106	1106	-	(Rocha, *et al.*, 2013)
108	515	0.770	2-Nonenal	$C_9H_{16}O$	18829-56-6	1162	-	1164	(Engel & Ratel, 2007)
109	545	0.700	4-Decenal	$C_{10}H_{18}O$	21662-09-9	1195	-	1193	(Adams, 2000)
110	555	0.630	Decanal	$C_{10}H_{20}O$	112-31-2	1207	1206	-	(Rocha, *et al.*, 2012)
111	565	0.990	2,4-Nonadienal	$C_9H_{14}O$	6750-03-4	1219	-	1212	(Lazari, *et al.*, 2000)
112	600	0.770	2-Decenal	$C_{10}H_{18}O$	2497-25-8	1260	-	1261	(Adams, 1995)
113	630	0.920	2,4-Decadienal (isomer)	$C_{10}H_{16}O$	2363-88-4	1295	-	1298	(Spadone, *et al.*, 1990)
114	640	0.620	Undecanal	$C_{11}H_{22}O$	112-44-7	1307	1306	-	(Rocha, *et al.*, 2012)
115	650	0.960	2,4-Decadienal (isomer)	$C_{10}H_{16}O$	25152-84-5	1320	-	1314	(Adams, 2000)
116	685	0.770	2-Undecenal	$C_{11}H_{20}O$	2463-77-6	1364	-	1376	(Ramarathnam, *et al.*, 1993)
117	720	0.650	Dodecanal	$C_{12}H_{24}O$	112-54-9	1407	1406	-	(Rocha, *et al.*, 2012)
118	730	1.010	2,4-Undecadienal	$C_{11}H_{18}O$	30361-29-6	1420	-	1416	(Lizárraga-Guerra, *et al.*, 1997)
119	805	0.680	Tridecanal	$C_{13}H_{26}O$	10486-19-8	1513	1512	-	(Rocha, *et al.*, 2012)
120	890	0.710	Tetradecanal	$C_{14}H_{28}O$	124-25-4	1613	1613	-	(Rocha, *et al.*, 2012)

			Aromatics						
121	335	1.550	Benzaldehyde	C_7H_6O	100-52-7	965	964	-	(Caldeira, *et al.*, 2011)
122	410	1.620	Benzeneacetaldehyde	C_8H_8O	122-78-1	1046	1049	-	(Xu, *et al.*, 2003)
123	410	1.860	2-Hydroxybenzaldehyde	$C_7H_6O_2$	90-02-8	1047	-	1041	(Adams, 1995)
124	435	1.360	2-Methylbenzaldehyde	C_8H_8O	529-20-4	1074	-	1067	(Pino, *et al.*, 2005)
125	445	1.410	4-Methylbenzaldehyde	C_8H_8O	104-87-0	1085	-	1079	(Pino, *et al.*, 2005)
126	515	1.640	2-Phenylpropenal	C_9H_8O	4432-63-7	1163	-	1148	(Shapi & Hesso, 1990)
127	565	1.360	3,5-Dimethylbenzaldehyde	$C_9H_{10}O$	5779-95-3	1219	-	-	-
128	585	1.140	4-(1-Methylethyl)-benzaldehyde	$C_{10}H_{12}O$	122-03-2	1243	-	1242	(Adams, *et al.*, 2005)
129	620	2.060	3-Phenyl-2-propenal	C_9H_8O	104-55-2	1285	1296	-	(Bieri & Marriott, 2008)
130	640	1.090	4-(t-Butyl)benzaldehyde	$C_{11}H_{14}O$	939-97-9	1308	-	-	-
131	820	1.030	Lily aldehyde	$C_{14}H_{20}O$	-	1531	-	-	-
			Esters						
			Aliphatics						
132	95	0.440	Ethyl acetate	$C_4H_8O_2$	141-78-6	601	-	608	(El-Sayed, *et al.*, 2005)
133	130	0.510	Ethyl propenoate	$C_5H_8O_2$	140-88-5	675	-	702	(de Souza, *et al.*, 2006)
134	135	0.530	Methyl 2-methylpropenoate	$C_5H_8O_2$	80-62-6	685	710	-	(Xu, *et al.*, 2003)
135	165	0.460	Ethyl isobutyrate	$C_6H_{12}O_2$	97-62-1	748	-	747	(Steinhaus & Schieberle, 2007)
136	175	0.500	Isobutyl ethanoate	$C_6H_{12}O_2$	110-19-0	769	-	770	(Guichard & Souty, 1988)
137	195	0.520	Ethyl butanoate	$C_6H_{12}O_2$	105-54-4	806	-	800	(Adams, 1995)
138	205	0.550	Butyl ethanoate	$C_6H_{12}O_2$	123-86-4	818	-	812	(Quijano, *et al.*, 2007)
139	235	0.510	2-Pentyl acetate	$C_7H_{14}O_2$	626-38-0	854	-	-	-
140	255	0.550	Isoamyl ethanoate	$C_7H_{14}O_2$	123-92-2	877	-	876	(Quijano, *et al.*, 2007)
141	255	0.730	1-Methoxy-2-propyl acetate	$C_6H_{12}O_3$	108-65-6	877	-	-	-
142	260	0.540	2-Methylbutyl acetate	$C_7H_{14}O_2$	624-41-9	883	-	880	(Pino, *et al.*, 2005)
143	315	0.640	Ethyl tiglate	$C_7H_{12}O_2$	5837-78-5	943	-	949	(Pino, *et al.*, 2005)
144	320	1.340	2-Methylamyl acetate	$C_9H_{20}O$	7789-99-3	949	-	-	-
145	330	0.510	Isobutyl butanoate	$C_8H_{16}O_2$	539-90-2	958	-	958	(Moio, *et al.*, 2000)
146	340	0.700	*m/z* 43, 71, 87, 59		-	969	-	-	-
147	365	0.540	Butyl butanoate	$C_8H_{16}O_2$	109-21-7	995	-	993	(Quijano, *et al.*, 2007)
148	365	0.620	3-Methylbutyl-2-propenoate	$C_8H_{14}O_2$	-	995	-	-	-
149	370	1.420	Ethylene diethanoate	$C_6H_{10}O_4$	111-55-7	1002	-	-	-
150	370	0.560	Ethyl hexanoate	$C_8H_{16}O_2$	123-66-0	1001	-	997	(Engel & Ratel, 2007)
151	375	0.440	Propyl pivalate	$C_8H_{16}O_2$	5129-35-1	1006	-	-	-
152	375	0.550	Pentyl propanate	$C_8H_{16}O_2$	624-54-4	1006	-	1006	(Bauchot, *et al.*, 1998)
153	385	1.100	Ethyl 2-methyl-3-oxopentanoate	$C_7H_{12}O_3$	17422-12-7	1018	-	-	-
154	410	0.530	Methyl 2-ethylhexanoate	$C_9H_{18}O_2$	816-19-3	1045	-	1043	(Fernando & Grün, 2001)
155	420	0.530	Isopentyl butanoate	$C_9H_{18}O_2$	106-27-4	1056	-	1054	(Quijano, *et al.*, 2007)
156	440	0.560	t-Butyl acetoacetate	$C_8H_{14}O_3$	1694-31-1	1078	-	-	-
157	455	0.740	2-Butoxyethyl acetate	$C_8H_{16}O_3$	112-07-2	1095	-	1096	(Pino, *et al.*, 2004)
158	460	0.550	Ethyl heptanoate	$C_9H_{18}O_2$	106-30-9	1101	-	1095	(Adams, 1995)
159	510	0.550	3-Methylheptyl acetate	$C_{10}H_{20}O_2$	72218-58-7	1156	-	-	-
160	520	1.230	3-Methylphenyl acetate	$C_9H_{10}O_2$	122-46-3	1168	-	-	-
161	545	0.560	Ethyl octanoate	$C_{10}H_{20}O_2$	106-32-1	1195	-	1195	(Adams, 1995)

162	560	0.920	Dimethyl 2,4-dimethylpentanedioate	$C_9H_{16}O_4$	2121-68-8	1213	-	-	-
163	575	0.580	2-Ethylhexyl 2-propenoate	$C_{11}H_{20}O_2$	103-11-7	1230	-	-	-
164	620	0.970	Methyl 2-phenylbutanoate	$C_{11}H_{14}O_2$	2294-71-5	1284	-	-	-
165	630	0.560	Ethyl nonanoate	$C_{11}H_{22}O_2$	123-29-5	1295	-	1294	(Pino, *et al.*, 2005)
166	630	0.600	4-tert-Butylcyclohexyl acetate (isomer)	$C_{12}H_{22}O_2$	-	1295	-	-	-
167	660	0.630	4-tert-Butylcyclohexyl acetate (isomer)	$C_{12}H_{22}O_2$	-	1332	-	-	-
168	665	1.120	Methyl 2-(phenylmethyl)prop-2-enoate	$C_{11}H_{12}O_2$	3070-71-1	1339	-	-	-
169	675	0.750	3-Phenyl-2-propenyl propionate	$C_{12}H_{14}O_2$	103-56-0	1351	-	-	-
170	690	0.650	4-tert-Butylcyclohexyl acetate (isomer)	$C_{12}H_{22}O_2$	32210-23-4	1370	-	1368	(Zellner, *et al.*, 2008)
171	695	0.920	3-hydroxy-2,4,4-trimethylpentyl 2-methyl-propanoate	$C_{12}H_{24}O3$	74367-34-3	1376	-	1381	(Kallio, *et al.*, 2006)
172	710	0.580	Ethyl decanoate	$C_{12}H_{24}O_2$	110-38-3	1395	-	1394	(Adams, 1995)
173	725	0.860	Decyl 2-methoxyacetate	$C_{13}H_{26}O_3$	259141-02-1	1414	-	-	-
174	760	0.970	Dibutyl-2-butenedioate	$C_{12}H_{20}O_4$	105-76-0	1458	-	-	-
175	760	1.080	Ethyl 2-phenylbutanoate	$C_{12}H_{16}O_2$	119-43-7	1458	-	-	-
176	775	0.840	Diisobutyl butanedioate	$C_{12}H_{22}O_4$	925-06-4	1476	-	-	-
177	790	0.830	Dimethylphenethyl butyrate	$C_{14}H_{20}O_2$	10094-34-5	1495	-	1488	(Wang, *et al.*, 2004)
178	800	1.090	5-Phenyl-2-pentenoic acid ethyl ester	$C_{13}H_{16}O_2$	55282-95-6	1507	-	-	-
179	905	0.600	Isopropyl dodecanoate	$C_{15}H_{30}O_2$	10233-13-3	1630	-	1618	(Wu, *et al.*, 2005)
180	960	0.550	3-Tridecanyl propionate	$C_{16}H_{32}O_2$	-	1695	-	-	-
181	985	0.650	4-Tridecanyl propionate	$C_{16}H_{32}O_2$	-	1724	-	-	-
182	1070	0.660	Isopropyl tetradecanoate	$C_{17}H_{34}O2$	110-27-0	1824	1801	-	(Silva, *et al.*, 2010)
			Aromatics						
183	460	1.210	Methyl benzoate	$C_8H_8O_2$	93-58-3	1101	-	1091	(Adams, 1995)
184	520	1.270	Benzyl ethanoate	$C_9H_{10}O_2$	140-11-4	1168	-	1163	(Adams, 1995)
185	525	1.050	Ethyl benzoate	$C_9H_{10}O_2$	93-89-0	1173	-	1170	(Adams, 1995)
186	545	1.050	Phenylethyl acetate	$C_{10}H_{12}O_2$	93-92-5	1196	-	1192	(Adams, 1995)
187	600	1.160	2-Phenylethyl acetate	$C_{10}H_{12}O_2$	103-45-7	1260	-	1256	(Adams, 1995)
188	615	1.190	Methyl benzenepropanoate	$C_{10}H_{12}O_2$	103-25-3	1278	-	1280	(Tzakou, *et al.*, 2000)
189	640	1.020	Phenylpropyl acetate	$C_{11}H_{14}O_2$	10402-52-5	1308	-	-	-
190	650	0.860	Dimethylphenethyl acetate	$C_{12}H_{16}O_2$	151-05-3	1320	-	-	-
191	655	0.910	Isobutyl benzoate	$C_{11}H_{14}O_2$	120-50-3	1326	-	1346	(Zaikin, 2010)
192	675	0.750	3-Phenyl-2-propenyl propionate	$C_{12}H_{14}O_2$	103-56-0	1351	-	-	-
193	695	0.970	4-Phenyl-2-butyl acetate	$C_{12}H_{16}O_2$	10415-88-0	1376	-	1398	(Zoghbi, *et al.*, 1999)
194	710	0.940	o-Methylbenzyl acetate	$C_{10}H_{12}O_2$	17373-93-2	1395	-	-	-
195	730	1.040	2-Methyl-4-phenyl-butyric acid, methyl ester	$C_{12}H_{16}O_2$	-	1420	-	-	-
196	790	0.820	Dimethyl benzyl carbinyl butyrate	$C_{14}H_{20}O_2$	10094-34-5	1495	-	1488	(Wang, *et al.*, 2004)
197	790	1.220	Methyl 5-phenylvalerate	$C_{12}H_{16}O_2$	20620-59-1	1495	-	-	-
198	795	1.110	Ethyl-5-phenyl-2-pentenoate	$C_{13}H_{16}O_2$	55282-95-6	1501	-	-	-
199	860	1.090	Amyl salicylate	$C_{12}H_{16}O_3$	2050-08-0	1578	-	-	-
			Cyclics						
200	560	1.660	Cyclopropanecarboxylic acid, 2-pentyl ester	$C_9H_{16}O_2$	-	1214	-	-	-
201	570	0.940	Cyclopropanecarboxylic acid, 2-ethylhexyl ester	$C_{12}H_{22}O_2$	103604-31-5	1225	-	-	-

			Ethers						
			Aliphatics						
202	120	0.470	2-Ethoxybutane	$C_6H_{14}O$	2679-87-0	654	-	622	(Zhao, *et al.*, 2008)
203	330	0.680	Vinyl (2-butoxy)ethyl ether	$C_8H_{16}O_2$	4223-11-4	959	-	-	-
204	435	0.530	Methoxycyclohexane	$C_7H_{14}O$	931-56-6	1073	-	-	-
205	725	0.860	1-(Methoxymethoxy)-hexane	$C_8H_{18}O_2$	66675-06-7	1414	-	-	-
			Aromatics						
206	390	0.910	1-Methoxy-3-methyl-benzene	$C_8H_{10}O$	100-84-5	1023	-	1028	(Larsen & Frisvad, 1995)
207	445	0.860	(2-Methoxyethyl)-benzene	$C_9H_{12}O$	3558-60-9	1084	-	1080	(Adams, 1995)
208	520	1.320	1,4-Dimethoxybenzene	$C_8H_{10}O_2$	150-78-7	1168	-	1163	(Adams, 1995)
209	535	0.710	[(1,1-Dimethylethoxy)methyl]-benzene	$C_{11}H_{16}O$	3459-80-1	1184	-	-	-
210	550	0.960	1-Methoxy-4-(2-propenyl)-benzene	$C_{10}H_{12}O$	140-67-0	1201	-	1195	(Adams, 2000)
211	715	1.270	1,1'-Oxybis-benzene	$C_{12}H_{10}O$	101-84-8	1402	-	1396	(Pino, *et al.*, 2005)
212	755	1.040	p-(Dimethoxymethyl)-tert-butylbenzene	$C_{13}H_{20}O_2$	-	1451	-	-	-
213	805	1.750	1-Methoxy-4-(4-methyl-4-pentenyl)-benzene	$C_{13}H_{18}O$	74672-06-3	1514	-	-	-
			Furan – type compounds						
214	95	0.420	2-Methylfuran	C_5H_6O	534-22-5	601	-	605	(Engel & Ratel, 2007)
215	105	0.400	Tetrahydrofuran	C_4H_8O	109-99-9	622	634	-	(Perestrelo, *et al.*, 2011)
216	130	0.460	2,5-Dimethylfuran	C_6H_8O	625-86-5	675	-	667	(Pino, *et al.*, 2002)
217	215	0.890	2-(Methoxymethyl)furan	$C_6H_8O_2$	13679-46-4	830	-	-	-
218	225	2.310	Furfural	$C_5H_4O_2$	98-01-1	844	-	835	(Methven, *et al.*, 2007)
219	265	0.510	2,5-Diethylfuran	$C_8H_{12}O$	-	889	-	-	-
220	270	0.550	2-Butylfuran	$C_8H_{12}O$	4466-24-4	895		894	(Engel & Ratel, 2007)
221	290	1.860	Acetylfuran	$C_6H_6O_2$	1192-62-7	918	911	-	(Xu, *et al.*, 2003)
222	310	0.660	2-Methyl-5-Isopropenylfuran	$C_8H_{10}O$	-	938	-	-	-
223	325	0.860	2-Methyl-5-(methylthio)furan	C_6H_8OS	13678-59-6	954	-	-	-
224	340	0.510	2-Butyltetrahydrofuran	$C_8H_{16}O$	1004-29-1	969	-	981	(Aaslyng, *et al.*, 1998)
225	365	0.560	2-Pentylfuran	$C_9H_{14}O$	3777-69-3	995	995	-	(Perestrelo, *et al.*, 2011)
226	370	1.220	Benzofuran	C_8H_6O	271-89-6	1001	1001	-	(Perestrelo, *et al.*, 2011)
227	455	0.560	2-Hexylfuran	$C_{10}H_{16}O$	3777-70-6	1095	1097	-	(Silva, *et al.*, 2010)
228	495	0.800	2-(1,1-Dimethylethyl)-4-methylfuran	$C_9H_{14}O$	6141-68-0	1140	-	-	-
229	550	1.230	2-Pentanoylfuran	$C_9H_{12}O_2$	-	1201	-	-	-
230	570	1.480	3-Phenylfuran	$C_{10}H_8O$	13679-41-9	1225	-	1228	(Solina, *et al.*, 2005)
231	735	0.830	4,5-Diethyl-2,3-dihydro-2,3-dimethylfuran	$C_{10}H_{18}O$	54244-89-2	1426	-	-	-
232	785	1.280	4-Hexyl-2,5-dihydro-2,5-dioxo-3-furanacetic acid	$C_{12}H_{16}O_5$	39212-21-0	1489	-	-	-
			Furanones						
233	425	1.270	4-Methoxy-2,5-dimethyl-3(2H)furanone	$C_7H_{10}O_3$	4077-47-8	1063	-	1065	(Pino, *et al.*, 2005)
234	455	1.060	1-(2,4-Dimethyl-furan-3-yl)ethanone	$C_8H_{10}O_2$	32933-07-6	1096	-	-	-
235	685	1.470	Dihydro-5-pentyl-2(3H)furanone	$C_9H_{16}O_2$	104-61-0	1364	-	1364	(Zhao, *et al.*, 2008)
236	770	1.520	5-Hexyldihydro-2(3H)furanone	$C_{10}H_{18}O_2$	706-14-9	1471	-	1470	(Pino, *et al.*, 2005)
			Halogenated compounds						

237	130	0.900	Bromodichloromethane	$CHBrCl_2$	75-27-4	676	664	-	(Rocha, *et al.*, 2013)
238	185	0.590	5-Chloro-2-pentanone	C_5H_9ClO	5891-21-4	791	-	-	-
239	200	0.480	Tetrachloroethylene	C_2Cl_4	127-18-4	812	816	-	(Xu, *et al.*, 2003)
240	230	0.780	Chlorobenzene	C_6H_5Cl	108-90-7	848	852	-	(Xu, *et al.*, 2003)
241	325	0.610	1-Bromo-2-propanone	C_3H_5BrO	598-31-2	953	-	-	-
242	380	0.960	1,4-Dichlorobenzene	$C_6H_4Cl_2$	106-46-7	1012	1015	-	(Xu, *et al.*, 2003)
243	380	1.200	Benzyl chloride	C_7H_7Cl	100-44-7	1012	1023	-	(Dallüge, *et al.*, 2002)
244	400	0.390	3-Chloro-1,1,2,2-tetramethyl-cyclopropane	$C_7H_{13}Cl$	14123-41-2	1034	-	-	-
245	425	0.520	Octyl chloride	$C_8H_{17}Cl$	111-85-3	1062	-	1064	(Engel & Ratel, 2007)
246	450	0.400	3-Trifluoroacetoxy-6-ethyldecane	$C_{14}H_{25}F_3O_2$	-	1089	-	-	-
247	450	0.500	N-(2-Fluorophenyl)octanamide	$C_{14}H_{20}FNO$	-	1089	-	-	-
248	480	1.150	(2-Chloroethenyl)-benzene	C_8H_7Cl	622-25-3	1124	-	-	-
249	520	1.530	3,4,5-Trichloropyridine	$C_5H_2Cl_3N$	33216-52-3	1168	-	-	-
250	555	0.710	5,7,7-Trichloro-6-hepten-2-one	$C_7H_9Cl_3O$	-	1207	-	-	-
251	565	0.740	1-Bromo-3,3-dimethyl-2-butanone	$C_6H_{11}BrO$	5469-26-1	1219	-	-	-
252	600	1.310	2,4-Dichlorobenzaldehyde	$C_7H_4Cl_2O$	874-42-0	1260	-	-	-
253	640	0.680	2-Bromo-2-methylbutane	$C_5H_{11}Br$	507-36-8	1307	-	-	-
254	650	0.570	1-Fluoro-decane	$C_{10}H_{21}F$	334-56-5	1320	-	-	-
255	675	0.420	3-Chloro-1,1,2,2-tetramethylcyclopropane	$C_7H_{13}Cl$	14123-41-2	1351	-	-	-
256	740	1.190	3-Benzyl-4-bromo-1,2,3-triazole 1-oxide	$C_9H_8BrN_3O$	-	1433	-	-	-
257	770	0.570	1-Chloroundecane	$C_{11}H_{23}Cl$	2473-03-2	1470	-	-	-
258	780	1.130	(2-Chloro-2,3-dimethylcyclopropyl)benzene	$C_{11}H_{13}Cl$	-	1483	-	-	-
259	855	1.340	2-Bromoisobutyrophenone	$C_{10}H_{11}BrO$	10409-54-8	1572	-	-	-
			Hydrocarbons						
			Aliphatics						
260	130	0.340	Heptane	C_7H_{16}	142-82-5	674	-	700	(Adams, 1995)
261	190	0.360	2,4-Dimethylheptane	C_9H_{20}	2213-23-2	800	819	-	(Rocha, *et al.*, 2013)
262	215	0.420	1,3-Octadiene	C_8H_{14}	1002-33-1	830	-	827	(Engel & Ratel, 2007)
263	275	0.370	Nonane	C_9H_{20}	111-84-2	900	900	-	(Caldeira, *et al.*, 2011)
264	365	0.370	C10 (*m/z* 57, 41, 39, 55)	-	-	995	-	-	-
265	370	0.380	Decane	$C_{10}H_{22}$	124-18-5	1000	1000	-	(Rocha, *et al.*, 2012)
266	375	0.380	C10 (*m/z* 57, 97, 41, 55)	$C_{12}H_{24}$	123-48-8	1006	-	-	-
267	400	0.390	3,4,4-Trimethyl-2-pentene	C_8H_{16}	598-96-9	1034	-	-	-
268	410	0.390	2,3,4-Trimethyl-2-pentene	C_8H_{16}	565-77-5	1045	-	-	-
269	410	0.710	Nonatetra-1,3,5,7-ene	C_9H_{12}	83829-35-0	1045	-	-	-
270	420	0.400	C10 (*m/z* 57, 41, 55, 69)	$C_{12}H_{24}$	7756-94-7	1056	-	-	-
271	420	0.740	C10 (*m/z* 41, 69, 39, 67)	C_8H_{14}	998-94-7	1056	-	-	-
272	425	1.060	C10 (*m/z* 41, 70, 39, 43)	C_8H_{16}	16106-59-5	1062	-	-	-
273	465	0.390	Undecane	$C_{11}H_{24}$	1120-21-4	1106	1101	-	(Silva, *et al.*, 2010)
274	465	0.650	1,9-Dodecadiene	$C_{12}H_{22}$	-	1106	-	-	-
275	520	0.890	7-Methyl-2-decene	$C_{11}H_{22}$	74630-23-2	1168	-	-	-
276	540	0.910	C12 (*m/z* 41, 55, 69, 43)	$C_{14}H_{28}$	-	1190	-	-	-
277	550	0.400	Dodecane	$C_{12}H_{26}$	112-40-3	1201	1201	-	(Silva, *et al.*, 2010)
278	635	0.410	Tridecane	$C_{13}H_{28}$	629-50-5	1301	1301	-	(Salvador, *et al.*, 2013)
279	715	0.420	Tetradecane	$C_{14}H_{30}$	629-59-4	1401	1400	-	(Rocha, *et al.*, 2012)

280	765	0.420	C14(*m/z* 43, 57, 41, 71)	$C_{15}H_{32}$	1560-95-8	1463	-	-	-
281	790	0.470	1-Pentadecene	$C_{15}H_{30}$	13360-61-7	1494	-	1492	(Flamini, *et al.*, 2005)
282	795	0.450	Pentadecane	$C_{15}H_{32}$	629-62-9	1501	1500	-	(Rocha, *et al.*, 2012)
283	880	0.490	Hexadecane	$C_{16}H_{34}$	544-76-3	1601	1600	-	(Rocha, *et al.*, 2012)
284	965	0.430	Heptadecane	$C_{17}H_{36}$	629-78-7	1701	1700	-	(Rocha, *et al.*, 2012)
285	1010	0.440	Octadecane	$C_{18}H_{38}$	593-45-3	1754	1800	-	(Rocha, *et al.*, 2012)
286	1150	0.450	Nonadecane	$C_{19}H_{40}$	629-92-5	1919	1900	-	(Rocha, *et al.*, 2012)
			Aromatics						
287	115	0.460	Benzene	C_6H_6	71-43-2	643	-	648	(Isidorov, *et al.*, 2003)
288	170	0.540	Toluene	C_7H_8	108-88-3	759	771		(Xu, *et al.*, 2003)
289	240	0.580	Ethylbenzene	C_8H_{10}	100-41-4	860	-	866	(Engel & Ratel, 2007)
290	250	0.590	1,3-Dimethylbenzene	C_8H_{10}	108-38-3	871	-	874	(Engel & Ratel, 2007)
291	270	0.640	1,2-Dimethylbenzene	C_8H_{10}	95-47-6	901	900	908	(Rocha, *et al.*, 2013)
292	300	0.570	(1-Methylethyl)benzene	C_9H_{12}	98-82-8	927	930	-	(Xu, *et al.*, 2003)
293	320	0.690	2-Propenylbenzene	C_9H_{10}	300-57-2	948	952	-	(Xu, *et al.*, 2003)
294	325	0.580	Propylbenzene	C_9H_{12}	103-65-1	953	959	-	(Xu, *et al.*, 2003)
295	330	0.470	*m/z* 91 106 77 119	-	-	958	-	-	-
296	335	0.590	1-Ethyl-4-methylbenzene	C_9H_{12}	622-96-8	964	970	-	(Xu, *et al.*, 2003)
297	350	0.630	1-Ethyl-2-methylbenzene	C_9H_{12}	611-14-3	980	988	-	(Xu, *et al.*, 2003)
298	355	0.750	(1-Methylethenyl)benzene	C_9H_{10}	98-83-9	985	988	-	(Xu, *et al.*, 2003)
299	365	0.640	1,3,5-Trimethylbenzene	C_9H_{12}	108-67-8	995	974	-	(Xu, *et al.*, 2003)
300	370	0.770	1-Propenylbenzene	C_9H_{10}	637-50-3	1001	-	1000	(Buchin, *et al.*, 2002)
301	390	0.580	1-Methyl-2-(1-methylethyl)benzene	$C_{10}H_{14}$	527-84-4	1023	-	1022	(Adams, 2000)
302	390	0.690	1,2,3-Trimethylbenzene	C_9H_{12}	526-73-8	1023	-	1022	(Adams, 2000)
303	420	0.590	1-Methyl-3-propylbenzene	$C_{10}H_{14}$	1074-43-7	1056	1058		(Xu, *et al.*, 2003)
304	425	0.610	2-Ethyl-1,4-dimethylbenzene	$C_{10}H_{14}$	1758-88-9	1062	-	1087	(Wang, *et al.*, 1994)
305	440	0.630	1-Ethyl-2,3-dimethylbenzene	$C_{10}H_{14}$	933-98-2	1079	1094	-	(Xu, *et al.*, 2003)
306	450	0.740	o-Isopropenyltoluene	$C_{10}H_{12}$	7399-49-7	1095	-	-	-
307	470	0.660	2-Ethyl-1,3-dimethylbenzene	$C_{10}H_{14}$	1758-88-9	1112	-	1087	(Wang, *et al.*, 1994)
308	505	0.750	1-Phenyl-1-butene	$C_{10}H_{12}$	1005-64-7	1151	-	-	-
309	480	0.670	1,2,4,5-Tetramethylbenzene	$C_{10}H_{14}$	95-93-2	1123	1130	-	(Xu, *et al.*, 2003)
310	485	0.970	Diethenylbenzene	$C_{10}H_{10}$	1321-74-0	1129	-	-	-
311	550	0.650	2,4-Diethyl-1-methylbenzene	$C_{11}H_{16}$	1758-85-6	1201	-	-	-
312	595	0.500	*m/z* 57, 175, 41, 91			1254	-	-	-
313	620	0.740	Pentamethylbenzene	$C_{11}H_{16}$	700-12-9	1283	-	1290	(Wang & Fingas, 1995)
314	700	1.270	Biphenyl	$C_{12}H_{10}$	92-52-4	1383	-	1385	(Leffingwell & Alford, 2005)
315	815	1.260	Bibenzyl	$C_{14}H_{14}$	103-29-7	1525	-	1519	(Song, *et al.*, 2003)
316	880	1.020	2-Methyl-6-phenyl-1,6-heptadiene	$C_{14}H_{18}$	51708-97-5	1601	-	-	-
317	910	0.590	(1,1-Diethylpropyl)benzene	$C_{13}H_{20}$	4170-84-7	1636	-	-	-
318	920	0.590	(1-Propylnonyl)benzene	$C_{18}H_{30}$	2719-64-4	1648	-	-	-
319	925	1.270	1,1'-(1,3-Propanediyl)bis-benzene	$C_{15}H_{16}$	1081-75-0	1654	-	1633	(Shapi & Hesso, 1990)
320	990	0.590	(1-Propylheptyl)benzene	$C_{16}H_{26}$	4537-12-6	1730	-	-	-
321	1005	0.610	(1-Pentylhexyl)benzene	$C_{17}H_{28}$	4537-14-8	1748	-	-	-
322	1140	0.770	(1-Methylnonyl)benzene	$C_{16}H_{26}$	4537-13-7	1907	-	-	-

			Cyclics						
323	100	0.340	Methylcyclopentane	C_6H_{12}	96-37-7	611	627	-	(Xu, *et al.*, 2003)
324	215	0.730	Dicyclopropylmethane	C_7H_{12}	5685-47-2	830	-	-	-
325	235	0.430	1-Ethylcyclohexene	C_8H_{14}	1453-24-3	853	-	-	-
326	270	0.530	1,2-Dimethyl-1,4-cyclohexadiene	C_8H_{12}	17351-28-9	895	-	-	-
327	270	0.790	1,3,5,7-Cyclooctatetraene	C_8H_8	629-20-9	895	-	894	(Wang & Guo, 2004)
328	305	0.490	1,2-Propadienylcyclohexane	C_9H_{14}	5664-17-5	932	-	-	-
329	350	0.540	1-Ethyl-1,4-cyclohexadiene	C_8H_{12}	19841-74-8	980	-	-	-
330	495	1.030	1-Cyclohexylheptene	$C_{13}H_{24}$	114614-83-4	1140	-	-	-
331	515	0.810	1-Ethyl-2-methylcyclohexane	C_9H_{18}	4923-78-8	1162	-	-	-
332	695	0.670	(2-Ethyl-1-methyl-1-butenyl)cyclohexane	$C_{13}H_{24}$	74810-42-7	1376	-	-	-
333	755	0.460	Isobutylcyclopentane	C_9H_{18}	3788-32-7	1451	-	-	-
334	840	0.480	Nonylcyclohexane	$C_{15}H_{30}$	2883-02-5	1554	-	1556	(Kenig, *et al.*, 2005)
			Ketones						
			Aliphatics						
335	75	0.390	2-Propanone (*m/z* 43, 58, 42, 39)	C_3H_6O	67-64-1	559	-	503	(Rembold, *et al.*, 1989)
336	90	0.440	2-Butanone	C_4H_8O	78-93-3	590	601	-	(Caldeira, *et al.*, 2011)
337	90	0.480	3-Buten-2-one	C_4H_6O	78-94-4	590	-	581	(Isidorov, *et al.*, 2006)
338	90	0.560	2,3-Butanedione	$C_4H_6O_2$	431-03-8	591	-	592	(Rychlik, 1998)
339	125	0.500	2-Pentanone	$C_5H_{10}O$	107-87-9	664	-	686	(Methven, *et al.*, 2007)
340	125	0.650	2,3-Pentanedione	$C_5H_8O_2$	600-14-6	665	-	693	(Engel & Ratel, 2007)
341	130	0.500	3-Pentanone	$C_5H_{10}O$	96-22-0	675	-	700	(Pino, *et al.*, 2005)
342	140	1.540	3-Hydroxy-2-butanone	$C_4H_8O_2$	513-86-0	698	733	-	(Salvador, *et al.*, 2013)
343	150	0.770	3-Penten-2-one	C_5H_8O	3102-33-8	717	-	735	(Pino, *et al.*, 2005)
344	160	0.510	3-Methyl-2-pentanone	$C_6H_{12}O$	565-61-7	738	750	-	(Xu, *et al.*, 2003)
345	190	0.690	4-Methyl-3-penten-2-one	$C_6H_{10}O$	141-79-7	801	801	-	(Rocha, *et al.*, 2013)
346	240	0.610	5-Methyl-2-hexanone	$C_7H_{14}O$	110-12-3	860	-	857	(Solina, *et al.*, 2005)
347	250	0.570	4-Heptanone	$C_7H_{14}O$	123-19-3	871	-	-	-
348	265	0.580	3-Heptanone	$C_7H_{14}O$	106-35-4	889	884	-	(Xu, *et al.*, 2003)
349	270	0.620	2-Heptanone	$C_7H_{14}O$	110-43-0	895	895	-	(Salvador, *et al.*, 2013)
350	275	0.740	5-Hepten-2-one	$C_7H_{12}O$	6714-00-7	901	-	866	(Moio & Addeo, 1998)
351	350	0.690	1-Octen-3-one	$C_8H_{14}O$	4312-99-6	980	977	-	(Xu, *et al.*, 2003)
352	355	0.710	2,3-Octanedione	$C_8H_{14}O_2$	585-25-1	985	-	980	(Engel & Ratel, 2007)
353	355	0.740	6-Methyl-5-hepten-2-one	$C_8H_{14}O$	110-93-0	985	985	-	(Xu, *et al.*, 2003)
354	360	0.600	3-Octanone	$C_8H_{16}O$	106-68-3	990	-	989	(Zhao, *et al.*, 2006)
355	360	0.640	2-Octanone	$C_8H_{16}O$	111-13-7	990	-	992	(Pino, *et al.*, 2005)
356	405	0.780	3-Octen-2-one	$C_8H_{14}O$	1669-44-9	1040	-	1046	(Qiming, *et al.*, 2006)
357	415	0.640	5-Ethyl-2-heptanone	$C_9H_{18}O$	-	1051	-	-	-
358	435	0.680	3-Nonen-2-one	$C_9H_{16}O$	14309-57-0	1073	-	1079	(Moio, *et al.*, 2000)
359	450	0.610	3-Nonanone	$C_9H_{18}O$	925-78-0	1090	-	1091	(Dickschat, *et al.*, 2004)
360	455	0.640	2-Nonanone	$C_9H_{18}O$	821-55-6	1095	1093	-	(Caldeira, *et al.*, 2011)
361	460	0.730	5-Nonen-2-one	$C_9H_{16}O$	27039-84-5	1101	-	-	-
362	470	1.040	6-Methyl-3,5-heptadiene-2-one	$C_8H_{12}O$	1604-28-0	1112	-	1110	(Jalali-Heravi, *et al.*, 2006)
363	490	0.630	*m/z* 43, 58, 71, 41	-	-	1134	-	-	-

364	495	0.760	3-Nonen-2-one (isomer)	$C_9H_{16}O$	18402-83-0	1140	-	1144	(Elmore, *et al.*, 2002)
365	530	0.690	1-Decen-3-one	$C_{10}H_{18}O$	-	1179	-	-	-
366	540	0.600	3-Decanone	$C_{10}H_{20}O$	928-80-3	1190	-	1186	(Adams, 1995)
367	545	0.640	2-Decanone	$C_{10}H_{20}O$	693-54-9	1195	1197	-	(Silva, *et al.*, 2010)
368	570	1.760	3,8-Nonadien-2-one	$C_9H_{14}O$	55282-90-1	1226	-	-	-
369	585	0.750	3-Decen-2-one	$C_{10}H_{18}O$	10519-33-2	1242	-	-	-
370	610	0.580	*m/z* 43, 58, 71, 72	-	-	1271	-	-	-
371	610	0.750	4,8-Dimethyl-nona-3,8-dien-2-one	$C_{11}H_{18}O$	-	1272	-	-	-
372	620	0.680	3-Undecen-2-one	$C_{11}H_{20}O$	-	1283	-	-	-
373	625	0.620	3-Undecanone	$C_{11}H_{22}O$	2216-87-7	1289	-	1283	(Smelcerovic, *et al.*, 2007)
374	625	0.720	5-Ethyl-4-methyl-5-hepten-3-one	$C_{10}H_{18}O$	74764-56-0	1289	-	-	-
375	630	0.640	2-Undecanone	$C_{11}H_{22}O$	112-12-9	1295	1291	-	(Silva, *et al.*, 2010)
376	650	0.560	5-Dodecanone	$C_{12}H_{24}O$	19780-10-0	1319	-	-	-
377	670	0.630	*m/z* 43, 58, 57, 71	-	-	1345	-	-	-
378	690	0.600	2-Methyl-5-undecanone	$C_{12}H_{24}O$	50639-02-6	1370	-	-	-
379	695	0.820	Tridecane-2,4-dione	$C_{13}H_{24}O_2$	25276-80-6	1376	-	-	-
380	710	0.680	2-Dodecanone	$C_{12}H_{24}O$	6175-49-1	1395	1398	-	(Silva, *et al.*, 2010)
381	735	0.590	6-Tridecanone	$C_{13}H_{26}O$	22026-12-6	1426	-	-	-
382	755	0.800	6,10-Dimethyl-,5,9-undecadien-2-one	$C_{13}H_{22}O$	3796-70-1	1451	-	1455	(Adams, *et al.*, 2005)
383	770	0.630	*m/z* 58, 43, 71, 57	-	-	1470	-	-	-
384	790	0.690	2-Tridecanone	$C_{13}H_{26}O$	593-08-8	1495	1498	-	(Silva, *et al.*, 2010)
			Aromatics						
385	435	1.500	Acetophenone	C_8H_8O	98-86-2	1074	1093	-	(Silva, *et al.*, 2010)
386	490	1.460	1-Phenyl-2-propanone	$C_9H_{10}O$	103-79-7	1135	-	1124	(Ferhat, *et al.*, 2007)
387	530	1.320	1-(4-Methylphenyl)-ethanone	$C_9H_{10}O$	122-00-9	1179	-	1182	(Adams, 1995)
388	595	1.140	1-Phenyl-1-butanone	$C_{10}H_{12}O$	495-40-9	1254	-	-	-
389	650	1.070	4-Isopropylacetophenone	$C_{11}H_{14}O$	645-13-6	1320	-	-	-
390	910	2.210	Benzophenone	$C_{13}H_{10}O$	119-61-9	1638	-	1621	(Pino, *et al.*, 2005)
			Cyclics						
391	270	0.890	Cyclohexanone	$C_6H_{10}O$	108-94-1	895	-	895	(Pino, *et al.*, 2005)
392	295	3.000	Butyrolactone	$C_4H_6O_2$	96-48-0	924	-	920	(Pino, *et al.*, 2004)
393	305	0.740	1-Cyclopentylethanone	$C_7H_{12}O$	6004-60-0	932	933	-	(Xu, *et al.*, 2003)
394	310	0.760	2-Ethylcyclopentanone	$C_7H_{12}O$	4971-18-0	938	-	-	-
395	405	0.750	Cyclooctanone	$C_8H_{14}O$	502-49-8	1040	-	-	-
396	495	0.740	2-n-Hexylcyclopentanone	$C_{11}H_{20}O$	13074-65-2	1140	-	-	-
397	515	0.790	3-Butylcyclopentanone	$C_9H_{16}O$	57283-81-5	1162	-	-	-
398	525	0.840	2-Ethylcycloheptanone	$C_9H_{16}O$	3183-41-3	1173	-	-	-
399	565	0.870	Cyclononanone	$C_9H_{16}O$	3350-30-9	1219	-	1239	(Dhanda, *et al.*, 2003)
400	605	0.780	3,3,5-Trimethylcyclohexanone	$C_9H_{16}O$	873-94-9	1266	-	1285	(Ventanas, *et al.*, 2008)
			N-compounds						
401	145	0.770	2-Nitropropane	$C_3H_7NO_2$	79-46-9	707	-	-	-
402	170	0.880	Pyridine	C_5H_5N	110-86-1	760	-	753	(Pino, *et al.*, 2005)

403	195	0.970	2-Methylpyridine	C_6H_7N	109-06-8	807	-	821	(Methven, *et al.*, 2007)
404	360	1.860	Benzonitrile	C_7H_5N	100-47-0	991	988	-	(Xu, *et al.*, 2003)
405	405	0.500	Cyclobutylamine	C_4H_9N	2516-34-9	1039	-	-	-
406	405	1.220	6-Nitro-2-hexene	$C_6H_{11}NO_2$	40244-96-0	1040	-	-	-
407	415	0.990	1-Nitrohexane	$C_6H_{13}NO_2$	646-14-0	1051	-	1050	(Solina, *et al.*, 2005)
408	445	0.710	3-Nitro-1-butene	$C_4H_7NO_2$	-	1084	-	-	-
409	490	1.470	N-Ethyl-benzenamine	$C_8H_{11}N$	103-69-5	1135	-	-	-
410	640	0.900	N,N-Dibutyl-formamide	$C_9H_{19}NO$	761-65-9	1307	-	1319	(Garcia-Esteban, *et al.*, 2004)
411	655	0.700	2,5-Dimethyl-4-nitro-3-hexanone	$C_8H_{15}NO_3$	59906-54-6	1326	-	-	-
412	955	0.810	2,4-di-t-butyl-6-nitro-phenol	$C_{14}H_{21}NO_3$	-	1689	-	-	-
413	1015	1.120	3-(4'-nitrophenyl)pentan-3-ol	$C_{11}H_{15}NO_3$	-	1760	-	-	-
			Pyrazines						
414	155	1.040	Pyrazine	$C_4H_4N_2$	290-37-9	729	-	734	(Methven, *et al.*, 2007)
415	215	1.000	2-Methylpyrazine	$C_5H_6N_2$	109-08-0	831	-	827	(Methven, *et al.*, 2007)
416	290	0.900	2,5-Dimethylpyrazine	$C_6H_8N_2$	123-32-0	917	-	911	(Adams, 1995)
417	295	0.920	Ethylpyrazine	$C_6H_8N_2$	13925-00-3	922	-	920	(Methven, *et al.*, 2007)
418	295	0.970	2,3-Dimethylpyrazine	$C_6H_8N_2$	5910-89-4	922	-	920	(Adams, 1995)
419	345	0.820	2-Isopropylpyrazine	$C_7H_{10}N_2$	29460-90-0	975	-	-	-
420	370	0.840	2-Ethyl-5-methylpyrazine	$C_7H_{10}N_2$	13360-64-0	1001	-	1000	(Solina, *et al.*, 2005)
421	370	0.870	Trimethylpyrazine	$C_7H_{10}N_2$	14667-55-1	1001	-	999	(Adams, 1995)
422	390	1.080	2-Ethenyl-5-methylpyrazine	$C_7H_8N_2$	13925-08-1	1023	-	1022	(Boylston & Viniyard, 1998)
423	420	0.750	2-Methyl-3-isopropylpyrazine	$C_8H_{12}N_2$	15986-81-9	1056	-	1056	(Gallois & Grimont, 1985)
424	440	0.760	3-Ethyl-2,5-dimethylpyrazine	$C_8H_{12}N_2$	13360-65-1	1079	-	1079	(Siegmund & Murkovic, 2004)
425	450	0.790	Tetramethylpyrazine	$C_8H_{12}N_2$	1124-11-4	1090	-	-	-
426	455	0.800	2-Methyl-5-propylpyrazine	$C_8H_{12}N_2$	29461-03-8	1095	-	-	-
427	470	0.860	2-Pentylpyrazine	$C_9H_{14}N_2$	-	1112	-	-	
428	500	0.740	2-Isobutyl-3-methylpyrazine	$C_9H_{14}N_2$	13925-06-9	1145	-	1144	(Risticevic, *et al.*, 2008)
429	515	0.690	3,5-Diethyl-2-methylpyrazine	$C_9H_{14}N_2$	18138-05-1	1162	-	1160	(Qian & Reineccius, 2003)
430	545	0.770	2-Butyl-3-methylpyrazine	$C_9H_{14}N_2$	15987-00-5	1195	-	-	
431	550	0.670	2,5-Dimethyl-3-isobutylpyrazine	$C_{10}H_{16}N_2$	32736-94-0	1201	-	1207	(Dickschat, *et al.*, 2005)
432	595	0.710	2-Butyl-3,5-dimethylpyrazine	$C_{10}H_{16}N_2$	50888-63-6	1254	-	1254	(Fan, *et al.*, 2007)
433	600	0.750	2-Methyl-3-propylpyrazine	$C_8H_{12}N_2$	15986-80-8	1260	-	-	
434	610	0.620	2,3-Diethyl-5-methylpyrazine	$C_9H_{14}N_2$	18138-04-0	1271	-	-	
435	615	0.630	2-(2-Methylpropyl)-3-(1-methylethyl)pyrazine	$C_{11}H_{18}N_2$	-	1277	-	-	
436	635	0.660	2,6-Dimethyl-3(2-methyl-1-butyl)pyrazine	$C_{11}H_{18}N_2$	56617-70-0	1301	-	1318	(Risticevic, *et al.*, 2008)
437	645	0.680	2,5-Dimethyl-3-(3-methylbutyl)pyrazine	$C_{11}H_{18}N_2$	18433-98-2	1313	-	1308	(Solina, *et al.*, 2005)
438	660	0.660	2,3,5-Trimethyl-6-butylpyrazine	$C_{11}H_{18}N_2$	10132-38-4	1332	-	-	
439	675	0.620	3,6-Dipropyl-2,5-dimethylpyrazine	$C_{12}H_{20}N_2$	-	1351	-	-	
440	680	0.630	1-(5-(2-Methyl-1-propyl)-2-pyrazinyl)-1-propanone	$C_{11}H_{16}N_2O$	86461-72-5	1357	-	-	
441	705	0.650	2-(3-Methylbutyl)-3,5,6-trimethylpyrazine	$C_{12}H_{20}N_2$	-	1388	-	-	
			S-compounds						
442	150	1.260	Thiazole	C_3H_3NS	288-47-1	718	-	735	(Methven, *et al.*, 2007)
443	155	0.620	Dimethyldisulfide	$C_2H_6S_2$	624-92-0	728	748	-	(Xu, *et al.*, 2003)

444	170	0.640	2-Methylthiophene	C_5H_6S	554-14-3	759	-	775	(Methven, *et al.*, 2007)
445	200	0.960	2-Methylthiazole	C_4H_5NS	3581-87-1	813	-	808	(Methven, *et al.*, 2007)
446	285	1.540	3-(Methylthio)-propanal	C_4H_8OS	3268-49-3	912	-	904	(Engel & Ratel, 2007)
447	340	0.920	Dimethyltrisulfide	$C_2H_6S_3$	3658-80-8	969	-	969	(Zhou, *et al.*, 2002)
448	390	1.850	2-Acetylthiazole	C_5H_5NOS	24295-03-2	1024	-	1014	(Adams, 1995)
449	425	0.650	1-(3-Thienyl)-2-propanone	C_7H_8OS	-	1062	-	-	-
450	445	1.310	Benzenemethanethiol	C_7H_8S	100-53-8	1085	-	1080	(Tellez, *et al.*, 2002)
451	480	1.530	2-Propionylthiazole	C_6H_7NOS	-	1124	-	-	-
452	520	1.080	[(Methylthio)methyl]-benzene	$C_8H_{10}S$	766-92-7	1168	-	1167	(Solina, *et al.*, 2005)
453	545	1.440	Benzo[b]thiophene	C_8H_6S	95-15-8	1196	-	1172	(Andersson & Weis, 1994)
454	575	1.960	Benzothiazole	C_7H_5NS	95-16-9	1232	1223	-	(Caldeira, *et al.*, 2011)
455	580	0.880	Cyclohexyl isothiocyanate	$C_7H_{11}NS$	1122-82-3	1236	-	-	-
456	590	0.660	2-Tertiobutylthiophene	$C_8H_{12}S$	-	1248	-	-	-
457	675	0.420	Cyclohexylmethylbutyl sulfite	$C_{11}H_{22}O_3S$	-	1351	-	-	-
458	680	0.540	S-Methyl(E)-oct-2-enethioate	$C_9H_{16}OS$	91944-66-0	1357	-	-	-
459	950	0.460	Cyclohexylmethyl hexyl sulfite	$C_{13}H_{26}O_3S$	-	1683	-	-	-
			Terpenic compounds						
			C_{10} Monoterpenenic compounds						
			Hydrocarbon-type						
460	310	0.410	α-Pinene	$C_{10}H_{16}$	7785-26-4	937	959	-	(Rocha, *et al.*, 2007)
461	330	0.480	Verbenene	$C_{10}H_{14}$	4080-46-0	958	963	-	(Petronilho S., *et al.*, 2013)
462	375	0.480	α-Phellandrene	$C_{10}H_{16}$	99-83-2	1006	1007	-	(Petronilho S., *et al.*, 2013)
463	395	0.500	Limonene	$C_{10}H_{16}$	138-86-3	1028	1027	-	(Petronilho S., *et al.*, 2013)
464	450	0.610	Bicyclo[3.2.1]oct-2-ene, 3-methyl-4-methylene-	$C_{10}H_{14}$	49826-53-1	1090	-	-	-
465	485	0.790	2,6-Dimethylbicyclo[3.2.1]octane	$C_{10}H_{18}$	-	1129	-	-	-
466	695	0.680	Bicyclo[3.1.1]heptane, 6,6-dimethyl-3-methylene-	$C_{10}H_{16}$	16022-04-1	1376	-	-	-
			Oxygen-containg compounds						
467	400	0.500	1,8-Cineole	$C_{10}H_{18}O$	470-82-6	1034	1041	-	(Petronilho S., *et al.*, 2013)
468	460	0.670	Dihydrolinalool	$C_{10}H_{22}O$	78-69-3	1101	-	1097	(Pino, *et al.*, 2005)
469	465	0.910	Linalool	$C_{10}H_{18}O$	78-70-6	1107	1107	-	(Petronilho S., *et al.*, 2013)
470	480	0.940	Fenchyl alcohol	$C_{10}H_{18}O$	1632-73-1	1123	1121	-	(Petronilho S., *et al.*, 2013)
471	500	1.090	Pinocarveol	$C_{10}H_{16}O$	547-61-5	1146	1148	-	(Petronilho S., *et al.*, 2013)
472	505	0.780	Camphor	$C_{10}H_{16}O$	464-48-2	1151	1147	-	(Jalali, *et al.*, 2012)
473	505	1.150	Verbenol	$C_{10}H_{16}O$	473-67-6	1151	-	1147	(Lo Presti, *et al.*, 2008)
474	510	0.670	p-Menthan-3-one	$C_{10}H_{18}O$	491-07-6	1156	1158	-	(Kallio, *et al.*, 2006)
475	520	0.840	Pinocarvone	$C_{10}H_{14}O$	30460-92-5	1168	1168	-	(Petronilho S., *et al.*, 2013)
476	525	1.120	Borneol	$C_{10}H_{18}O$	507-70-0	1174	1172	-	(Jalali, *et al.*, 2013)
477	530	0.920	Menthol	$C_{10}H_{20}O$	2216-51-5	1179	-	1172	(Sefidkon & Jamzad, 2005)
478	540	1.660	p-Cymen-8-ol	$C_{10}H_{14}O$	1197-01-9	1191	1203	-	(Jalali, *et al.*, 2013)
479	545	0.910	Dihydrocitronellol	$C_{10}H_{22}O$	106-21-8	1196	-	1196	(Stashenko, *et al.*, 2003)
480	545	1.020	α-Terpineol	$C_{10}H_{18}O$	98-55-5	1196	1201	-	(Petronilho S., *et al.*, 2013)
481	550	0.870	Myrtenal	$C_{10}H_{14}O$	564-94-3	1201	1204	-	(Jalali, *et al.*, 2013)
482	560	1.040	Verbenone	$C_{10}H_{14}O$	1196-01-6	1213	-	1204	(Quijano, *et al.*, 2007)

483	595	0.620	Linalyl acetate	$C_{12}H_{20}O_2$	115-95-7	1254	-	1257	(Quijano, *et al.*, 2007)
484	625	0.630	Endobornyl acetate	$C_{12}H_{20}O_2$	76-49-3	1289	-	1285	(Adams, 1995)
485	675	0.680	β-Terpenyl acetate	$C_{12}H_{20}O_2$	10198-23-9	1351	-	-	-
486	700	0.730	Geraniol acetate	$C_{12}H_{20}O_2$	105-87-3	1382	-	1383	(Adams, 1999)
487	735	0.910	Verdyl acetate	$C_{12}H_{16}O_2$	5413-60-5	1426	-	-	-
			C_{15} Sesquiterpenes						
			Hydrocarbon-type						
488	725	0.530	Valencene	$C_{15}H_{24}$	4630-07-3	1413	-	-	-
489	725	0.540	Longifolene	$C_{15}H_{24}$	475-20-7	1413	1395	-	(Jalali, *et al.*, 2013)
490	820	0.600	δ-Cadinene	$C_{15}H_{24}$	483-76-1	1530	1511	-	(Jalali, *et al.*, 2013)
491	820	0.690	Calamenene	$C_{15}H_{22}$	483-77-2	1530	1525	-	(Petronilho, *et al.*, 2011)
492	820	0.830	α-Bisabolene	$C_{15}H_{24}$	29837-07-8	1530	1537	-	(Petronilho S., *et al.*, 2013)
493	835	0.770	α-Calacorene	$C_{15}H_{20}$	21391-99-1	1548	1542	-	(Petronilho, *et al.*, 2011)
494	840	0.870	Patchulane	$C_{15}H_{26}$	19078-35-4	1554	-	-	-
495	1100	0.670	4,5,9,10-Dehydro-isolongifolene	$C_{15}H_{20}$	-	1860	-	-	-
			Oxygen-containg compounds						
496	850	0.920	Nerolidol	$C_{15}H_{26}O$	7212-44-4	1566	1568	-	(Jalali, *et al.*, 2013)
497	855	0.960	Longicamphenylone	$C_{14}H_{22}O$	-	1572	-	-	-
498	880	0.780	Torreyol	$C_{15}H_{26}O$	19435-97-3	1601	-	1618	(Marongiu, *et al.*, 2003)
499	890	1.000	Cedrol	$C_{15}H_{26}O$	77-53-2	1613	1600	-	(Petronilho S., *et al.*, 2013)
500	920	1.020	τ-Cadinol	$C_{15}H_{26}O$	5937-11-1	1648	1637	-	(Petronilho, *et al.*, 2011)
501	1175	0.820	α-Bisabolene epoxide	$C_{15}H_{24}O$	-	1951	-	-	-
			C_{13} Norisoprenoid						
502	575	1.080	Tetrahydroionol	$C_{13}H_{26}O$	4361-23-3	1231	-	-	-
503	645	0.610	Edulan	$C_{13}H_{20}O$	41678-30-2	1313	-	-	-
504	780	0.750	α-iso-methyl ionone	$C_{14}H_{22}O$	127-51-5	1482	-	-	-

[a] Retention times for first (^1t_R) and second (^2t_R) dimensions in seconds.
[b] RI: Retention Index obtained through the modulated chromatogram.
[c] RI: Retention Index reported in the literature for Equity-5 column or equivalents.

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Table S3 – GC peak areas of the 504 volatile compounds identified, by HS-SPME/GC×GC-ToFMS, from *A. niger* cultures in YGC medium (solid and liquid), at different growth times (3 and 5 days) and growth temperatures (25 and 37 °C).

Peak number	^1t_R[a] (s)	^2t_R[a] (s)	Compound	Formula	CAS	Solid medium				Liquid medium			
						25 ºC		37 ºC		25 ºC		37 ºC	
						3º day	5º day	3º day	5º day	3º day	5º day	3º day	5º day
						Peak area[b] ($x10^2$) and RSD (%)							
			Acids										
1	185	0.550	2-Methylpropanoic acid	$C_4H_8O_2$	79-31-2	-	-	-	-	-	0.17 (24)	-	-
2	200	1.860	Butanoic acid	$C_4H_8O_2$	107-92-6	-	-	-	-	20.01 (18)	-	9.47 (47)	-
3	240	1.130	3-Methylbutanoic acid	$C_5H_{10}O_2$	503-74-2	-	-	-	-	3.12 (27)	-	5.40 (32)	-
4	255	0.410	2-Methylbutanoic acid	$C_5H_{10}O_2$	116-53-0	-	-	-	-	16.76 (13)	0.19 (12)	21.62 (12)	-
5	485	3.370	2-Ethylhexanoic acid	$C_8H_{16}O_2$	149-57-5	1.17 (36)	3.29 (39)	-	-	-	-	-	-
6	535	3.680	Octanoic acid	$C_8H_{16}O_2$	124-07-2	1.51 (41)	8.01 (25)	-	-	11.92 (66)	-	-	0.06 (52)
7	615	3.200	Nonanoic acid	$C_9H_{18}O_2$	112-05-0	11.04 (33)	25.13 (29)	-	-	-	0.28 (38)	8.01 (35)	0.21 (36)
8	695	2.890	Decanoic acid	$C_{10}H_{20}O_2$	334-48-5	-	-	-	-	-	0.16 (22)	-	-
9	1020	2.650	Tetradecanoic acid	$C_{14}H_{28}O_2$	544-63-8	-	-	-	0.10 (27)	7.64 (43)	-	10.81 (35)	0.17 (38)
10	1105	2.590	Pentadecanoic acid	$C_{15}H_{30}O_2$	1002-84-2	-	-	-	0.06 (42)		-	-	-
					Subtotal (GC Peak Area)	13.72	36.43	-	0.16	59.45	0.80	55.32	0.44
					Subtotal (%)	0.22	0.35	-	0.45	1.01	0.79	0.65	0.56
			Alcohols										
			Aliphatics										
11	85	0.700	1-Propanol	C_3H_8O	71-23-8	6.33 (25)	2.74 (11)	0.07 (32)	-	3.19 (42)	0.05 (5)	9.08 (4)	0.06 (14)
12	95	0.640	2-Butanol	$C_4H_{10}O$	78-92-2	2.47 (42)	5.80 (47)	0.08 (35)	-	9.34 (7)	0.13 (24)	16.57 (39)	0.35 (21)
13	100	0.800	2-Methyl-1-propanol	$C_4H_{10}O$	78-83-1	162.12 (9)	202.84 (44)	0.31 (16)	-	284.01 (4)	7.29 (9)	498.74 (20)	5.14 (15)
14	115	0.910	1-Butanol	$C_4H_{10}O$	71-36-3	6.06 (24)	2.55 (38)		0.01 (9)	59.47 (30)	0.78 (23)	85.70 (16)	0.78 (15)
15	130	0.740	3-Pentanol	$C_5H_{12}O$	584-02-1	-			-	0.38 (20)	-	0.52 (38)	0.01 (21)
16	130	0.770	2-Pentanol	$C_5H_{12}O$	6032-29-7	0.24 (36)	0.59 (43)		-	1.33 (27)	0.02 (6)	4.00 (9)	0.04 (26)
17	145	1.260	3-Methyl-3-buten-1-ol	$C_5H_{10}O$	763-32-6	1.64 (36)			-	3.16 (24)	0.09 (30)	5.43 (21)	0.05 (9)
18	150	1.160	3-Methyl-1-butanol	$C_5H_{12}O$	123-51-3	429.19 (27)	1047.35 (39)	0.21 (17)	0.03 (40)	466.28 (36)	10.96 (8)	1495.88 (29)	19.77 (21)
19	165	0.800	4-Methyl-2-pentanol	$C_6H_{14}O$	108-11-2	-				0.15 (12)	-	0.26 (19)	-
20	170	0.770	2-Methyl-3-pentanol	$C_6H_{14}O$	565-67-3	-				0.97 (22)	0.01 (5)	1.01 (29)	0.01 (32)
21	170	1.120	1-Pentanol	$C_5H_{12}O$	71-41-0	2.25 (14)	3.90 (19)	1.08 (30)	0.14 (10)	3.18 (20)	-	7.00 (7)	0.06 (8)
22	180	1.490	3-Methyl-2-buten-1-ol	$C_5H_{10}O$	556-82-1	-	-	-	-	-	-	2.21 (22)	-
23	185	0.860	3-Methyl-2-pentanol	$C_6H_{14}O$	565-60-6	-	-	-	-	-	-	0.64 (18)	-
24	190	0.820	3-Hexanol	$C_6H_{14}O$	623-37-0	-	-	-	-	0.36 (33)	-	-	-
25	225	1.130	4-Methyl-1-pentanol	$C_6H_{14}O$	626-89-1	-	-	-	-	-	-	6.87 (13)	-
26	245	1.400	4-Methyl-3-penten-1-ol	$C_6H_{12}O$	763-89-3	-	-	-	-	-	-		0.04 (13)
27	255	1.140	1-Hexanol	$C_6H_{14}O$	111-27-3	2.84 (16)	2.19 (41)	0.09 (26)	0.01 (11)	2.70 (19)	0.03 (21)	4.77 (12)	0.04 (18)
28	255	1.480	4-Hexen-1-ol	$C_6H_{12}O$	928-92-7	4.59 (22)	-	1.02 (26)	0.40 (21)	-	-	-	-
29	260	1.100	1-Hepten-3-ol	$C_7H_{14}O$	4938-52-7	3.75 (8)	6.01 (30)	0.27 (28	0.03 (8)	-	-	-	-
30	270	0.820	4-Heptanol	$C_7H_{16}O$	589-55-9	-	-	-	-	-	-	2.30 (15)	-
31	275	0.840	3-Heptanol	$C_7H_{16}O$	589-82-2	0.57 (22)	0.83 (58)	0.03 (7)	0.0044 (7)	1.44 (16)	0.03 (10)	0.64 (21)	-
32	280	0.890	2-Heptanol	$C_7H_{16}O$	543-49-7	0.26 (22)	-	-	-	-	0.04 (8)	31.60 (35)	0.23 (40)
33	310	1.140	2-Hepten-1-ol (isomer)	$C_7H_{14}O$	55454-22-3	-	-	-	-	-		0.92 (43)	-
34	315	0.840	Butoxypropanol	$C_7H_{16}O_2$	5131-66-8	0.99 (32)	-	0.05 (10)	0.02 (5)	-	0.01 (29)	-	-
35	330	1.150	5-Methyl-1-hepten-4-ol	$C_8H_{16}O$	99328-46-8	-	-	-	-	-	-	0.89 (14)	-
36	335	0.870	2-Octanol	$C_8H_{18}O$	123-96-6	-	-	-	-	1.97 (29)	0.01 (19)	-	-
37	345	1.100	1-Heptanol	$C_7H_{16}O$	111-70-6	4.62 (22)	6.81 (36)	0.19 (12)	0.02 (31)	7.00 (68)	0.07 (13)	7.82 (20)	0.07 (56)
38	345	1.380	2-Hepten-1-ol	$C_7H_{14}O$	33467-76-4		1.04 (47)	0.05 (8)	0.01 (39)	-	-	-	-
39	350	1.050	1-Octen-3-ol	$C_8H_{16}O$	3391-86-4	1218.13 (17)	1087.31 (19)	82.08 (46)	7.13 (37)	97.21 (14)	2.32 (21)	3.36 (34)	0.04 (21)
40	350	1.210	Octa-1,5-dien-3-ol	$C_8H_{14}O$	50306-18-8	97.53 (39)	171.28 (21)	0.13 (34)	0.02 (15)	20.28 (10)	0.58 (11)	-	0.01 (28)
41	355	0.930	5-Octen-3-ol	$C_8H_{16}O$	-	-	-	-	-	58.22 (9)	0.87 (10)	-	-
42	365	1.010	6-Methyl-1-heptanol	$C_8H_{18}O$	1653-40-3	-	-	-	-	-	-	3.96 (40)	0.17 (53)

43	365	1.030	6-Methyl-5-hepten-2-ol	$C_8H_{16}O$	1569-60-4	9.35 (2)	-	1.04 (3)	0.03 (17)	-	0.08 (0)	5.18 (20)	0.26 (22)
44	365	1.270	3-Octanol	$C_8H_{18}O$	589-98-0	888.57 (25)	809.07 (30)	40.87 (28)	6.37 (17)	349.97 (10)	7.38 (7)	9.18 (41)	0.05 (26)
45	390	1.100	3-Ethyl-4-methyl-1-pentanol	$C_8H_{18}O$	100431-87-6	-	-	-	-	1.25 (13)	-	1.76 (44)	-
46	395	0.990	2-Ethyl- 1-hexanol	$C_8H_{18}O$	104-76-7	50.38 (30)	150.51 (47)	5.54 (58)	2.22 (7)	68.99 (24)	2.84 (21)	45.88 (7)	0.75 (19)
47	405	1.040	4-Methyl-1-heptanol	$C_8H_{18}O$	817-91-4	-	-	-	-	-	-	-	0.03 (44)
48	415	1.030	5-Methyl-1-heptanol	$C_8H_{18}O$	7212-53-5	-	5.62 (27)	-	-	-	-	-	-
49	435	0.790	2,6-Dimethyl-7-octen-2-ol	$C_{10}H_{20}O$	18479-58-8	6.51 (43)	12.30 (45)	0.44 (44)	0.10 (23)	8.38 (14)	0.12 (37)	5.39 (49)	0.17 (42)
50	435	1.280	2-Octen-1-ol	$C_8H_{16}O$	18409-17-1	167.54 (27)	322.12 (43)	8.11 (16)	0.42 (2)	1.30 (27)	-	-	-
51	440	0.740	1-Nonen-3-ol	$C_9H_{18}O$	21964-44-3	1.46 (26)	3.14 (17)	-	-	-	-	-	-
52	440	1.030	1-Octanol	$C_9H_{18}O_2$	111-87-5	24.78 (38)	48.15 (38)	3.00 (56)	0.12 (19)	3.18 (29)	0.35 (11)	10.76 (16)	0.19 (45)
53	445	0.700	2,6-Dimethyl-1,7-octadien-3-ol	$C_{10}H_{18}O$	22460-59-9	-	-	-	-	55.55 (34)	-	-	-
54	460	0.700	2,6-Dimethyl-2-octanol	$C_{10}H_{22}O$	18479-57-7	-	-	-	0.02 (18)	0.82 (27)	-	-	-
55	460	0.840	2-Nonanol	$C_9H_{20}O$	628-99-9	-	-	-	0.0024 (28)	10.61 (12)	0.50 (19)	50.13 (11)	0.63 (24)
56	465	0.990	7-Octen-2-ol	$C_8H_{16}O$	39546-75-3	-	-	-	-	6.07 (13)	0.28 (15)	50.23 (22)	0.53 (33)
57	480	1.030	2-Nonen-1-ol (isomer)	$C_9H_{18}O$	31502-14-4	1.36 (43)	-	-	-	-	-	-	-
58	490	1.010	1-Octen-4-ol	$C_8H_{16}O$	40575-42-6	0.59 (46)	-	-	0.01 (33)	-	-	-	-
59	505	1.040	2-Nonen-1-ol (isomer)	$C_9H_{18}O$		-	-	-	-	15.32 (18)	0.50 (40)	45.88 (14)	0.39 (22)
60	525	0.970	1-Nonanol	$C_9H_{20}O$	143-08-8	12.28 (36)	-	-	-	-	-	8.80 (42)	0.29 (47)
61	530	1.110	2-Nonen-1-ol (isomer)	$C_9H_{18}O$	22104-79-6	-	-	-	-	-	-	51.04 (32)	0.43 (39)
62	545	0.760	3-Decanol	$C_{10}H_{22}O$	1565-81-7	-	-	-	-	0.25 (36)	0.01 (39)	1.48 (48)	0.03 (35)
63	545	1.360	2-(2-Butoxyethoxy)-ethanol	$C_8H_{18}O_3$	112-34-5	2.68 (20)	-	-	0.02 (15)	0.98 (22)	-	-	-
64	565	0.860	2-Propyl-1-heptanol	$C_{10}H_{22}O$	10042-59-8	6.87 (42)	-	-	0.11 (32)	-	0.09 (22)	19.78 (34)	0.25 (40)
65	615	0.620	2-Decen-1-ol	$C_{10}H_{20}O$	18409-18-2	7.60 (35)	8.25 (29)	-	-	-	0.11 (15)		0.10 (16)
66	615	0.910	1-Decanol	$C_{10}H_{22}O$	112-30-1	4.83 (35)	21.22 (23)	0.70 (45)	-	-	-	34.12 (17)	0.05 (31)
67	625	0.730	4-Undecanol	$C_{11}H_{24}O$	4272-06-4	-	-	-	-	-	-	2.26 (34)	-
68	630	0.700	2,4-Undecadien-1-ol	$C_{11}H_{20}O$	77657-78-4	1.24 (18)	4.98 (37)	-	-	-	-		-
69	635	0.770	2-Undecanol	$C_{11}H_{24}O$	1653-30-1	0.60 (30)	1.03 (23)	-	-	-	-	0.79 (31)	-
70	645	0.830	2-Butyl-1-octanol	$C_{12}H_{26}O$	3913-02-8	-	2.28 (66)	-	0.02 (36)	-	-		-
71	725	0.860	2-Dodecanol	$C_{12}H_{26}O$	10203-28-8	-	-	-	-	3.19 (4)	-	0.69 (38)	-
72	760	0.960	6,10-Dimethyl-5,9-undecadien-2-ol	$C_{13}H_{24}O$	53837-34-6	2.31 (26)	-	-	-	-	-		-
73	775	0.940	1-Dodecanol	$C_{12}H_{26}O$	112-53-8	-	61.39 (46)	7.55 (26)	0.23 (22)	26.95 (17)	0.28 (30)	19.91 (45)	-
74	950	0.970	1-Tetradecanol	$C_{14}H_{30}O$	112-72-1	-	-	-	-	3.31 (35)	-	-	-
			Aromatics										
75	410	4.130	Benzyl alcohol	C_7H_8O	100-51-6	-	-	-	-	4.07 (14)	-	-	-
76	430	2.770	Methylbenzenemethanol	$C_8H_{10}O$	1445-91-6	-	-	-	-	2.47 (16)	-	3.88 (13)	0.03 (16)
77	450	1.990	2-Phenylisopropanol	$C_9H_{12}O$	617-94-7	1.11 (35)	1.95 (42)	0.08 (17)	0.02 (22)	1.29 (17)	-	0.95 (10)	0.02 (30)
78	455	1.070	4-Ethyl-1,3-benzenediol	$C_8H_{10}O_2$	2896-60-8	0.46 (36)	0.44 (32)	-	0.01 (38)	0.71 (33)	-	0.65 (11)	
79	475	3.030	2-Phenylethanol	$C_8H_{10}O$	60-12-8	1199.03 (48)	3198.84 (48)	0.93 (26)	0.04 (24)	385.48 (14)	2.63 (22)	1080.96 (19)	7.90 (15)
80	495	1.940	Methylbenzeneethanol	$C_9H_{12}O$	698-87-3	-	-	-	-	0.79 (20)	-	1.45 (14)	0.01 (15)
81	555	2.590	2,4,6-Trimethylphenol	$C_9H_{12}O$	527-60-6	3.03 (19)	-	0.55 (48)	0.02 (39)	1.90 (48)	-	-	-
82	680	2.700	2-(1,1-Dimethylethyl)-4-methylphenol	$C_{11}H_{16}O$	2409-55-4	-	1.60 (18)	-	-	-	-	-	-
83	805	2.060	2,4-bis(1,1-dimethylethyl)-phenol	$C_{14}H_{22}O$	96-76-4	23.78 (22)	96.43 (41)	1.18 (50)	0.42 (13)	52.01 (9)	0.52 (39)	35.47 (23)	0.15 (16)
84	810	0.780	Butyl hydroxy toluene	$C_{15}H_{24}O$	128-37-0	69.66 (41)	128.13 (31)	3.76 (44)	1.05 (45)	51.94 (17)	1.34 (27)	154.88 (36)	3.00 (35)
85	890	3.240	(1,1,3,3-tetramethylbutyl)-phenol	$C_{14}H_{22}O$	27193-28-8	0.85 (23)	-	-	-	-	-	-	
			Cyclics										
86	165	1.470	Cyclopropaneethanol	$C_5H_{10}O$	2566-44-1	-	-	-	-	-	-	1.92 (20)	-
87	545	1.150	3-Methylcyclohexanol	$C_7H_{14}O$	591-23-1	-	-	-	-	27.82 (35)	-	41.69 (25)	0.13 (28)
					Subtotal (GC Peak Area)	4430.47	7418.70	159.41	19.02	2105.24	40.32	3875.29	42.23
					Subtotal (%)	72.13	71.52	59.33	52.97	35.89	39.94	45.58	53.79
			Aldehydes										
			Aliphatics										
88	70	0.340	Acetaldehyde (*m/z* 44, 43, 42, 41)	C_2H_4O	75-07-0	41.67 (10)	48.26 (59)	0.78 (3)	0.10 (38)	17.59 (49)	0.52 (19)	17.29 (16)	0.66 (27)
89	110	0.460	3-Methylbutanal	$C_5H_{10}O$	590-86-3	18.93 (42)	36.83 (37)	0.39 (30)	0.04 (38)	73.46 (40)	2.01 (34)	88.10 (35)	1.94 (29)
90	110	0.650	2-Butenal	C_4H_6O	4170-30-3	0.96 (31)	0.76 (39)	0.05 (37)	0.0040 (41)	0.81 (30)	-	0.95 (48)	-
91	115	0.440	2-Methylbutanal	$C_5H_{10}O$	96-17-3	-	-	-	0.03 (24)	-	-	-	-
92	120	0.490	*m/z* 55, 84, 39, 56	C_5H_8O	-	-	-	-	0.01 (33)	1.92 (45)	-	-	0.01 (12)
93	125	0.500	Pentanal	$C_5H_{10}O$	110-62-3	2.58 (41)	3.35 (9)	0.18 (37)	0.01 (43)	-	-	-	-

94	155	0.670	2-Methyl-2-butenal	C_5H_8O	497-03-0	1.38 (26)	-	-	-	5.05 (29)	0.05 (22)	3.10 (43)	-
95	190	0.590	Hexanal	$C_6H_{12}O$	66-25-1	46.41 (20)	46.77 (18)	0.71 (8)	0.08 (17)	11.50 (13)	0.25 (10)	13.21 (20)	0.28 (48)
96	195	0.710	3-Hexenal	$C_6H_{10}O$	6789-80-6	15.72 (17)	6.78 (45)	2.08 (51)	0.14 (41)	1.12 (38)	-	-	-
97	275	0.620	Heptanal	$C_7H_{14}O$	111-71-7	13.38 (29)	18.27 (29)	0.56 (32)	0.13 (41)	21.26 (7)	0.39 (35)	18.70 (15)	0.44 (46)
98	325	0.560	2-Ethylhexanal	$C_8H_{16}O$	123-05-7	-	-	-	0.0043 (36)	-	-	-	-
99	330	0.790	2-Heptenal	$C_7H_{12}O$	18829-55-5	4.25 (24)	5.40 (21)	0.10 (31)	0.01 (17)	-	-	-	-
100	360	0.680	5-Methyl-2-heptenal	$C_8H_{14}O$	94705-03-0	-		0.09 (32)	-	-	-	-	-
101	370	0.640	Octanal	$C_8H_{16}O$	124-13-0	-	106.91 (8)	4.81 (11)	0.88 (9)	71.63 (24)	0.76 (30)	21.10 (41)	0.32 (26)
102	415	0.740	2-Octenal (isomer)	$C_8H_{14}O$	2363-89-5	1.60 (12)	4.56 (68)	0.07 (45)	0.03 (33)	-	-	-	-
103	420	0.650	2,6-Dimethyl-5-heptenal	$C_9H_{16}O$	106-72-9	-	-	0.04 (5)	-	-	-	-	-
104	425	0.780	2-Octenal (isomer)	$C_8H_{14}O$	2548-87-0	19.78 (15)	49.68 (38)	3.28 (41)	0.17 (37)	2.93 (14)	0.02 (50)	-	-
105	425	0.910	2,6-Octadienal	$C_8H_{12}O$	76917-23-2	2.34 (25)	-	0.60 (49)	-	-	-	-	-
106	430	0.640	2-Methylenehexanal	$C_7H_{12}O$	1070-66-2	-	-	-	0.01 (36)	-	-	-	-
107	465	0.630	Nonanal	$C_9H_{18}O$	124-19-6	144.30 (28)	180.11 (35)	6.04 (50)	0.72 (27)	57.58 (26)	3.93 (42)	185.31 (35)	1.60 (16)
108	515	0.770	2-Nonenal	$C_9H_{16}O$	18829-56-6	21.39 (15)	31.62 (12)	0.47 (39)	0.05 (26)	-	-	-	-
109	545	0.700	4-Decenal	$C_{10}H_{18}O$	21662-09-9	4.24 (37)	5.79 (19)	-		-	-	-	-
110	555	0.630	Decanal	$C_{10}H_{20}O$	112-31-2	93.75 (38)	125.35 (44)	2.68 (37)	0.14 (41)	46.82 (32)	1.85 (34)	115.47 (48)	1.65 (46)
111	565	0.990	2,4-Nonadienal	$C_9H_{14}O$	6750-03-4	0.90 (39)	-	-	-				
112	600	0.770	2-Decenal	$C_{10}H_{18}O$	2497-25-8	9.08 (41)	27.86 (35)	-	-	15.73 (30)	0.10 (47)	9.80 (8)	
113	630	0.920	2,4-Decadienal (isomer)	$C_{10}H_{16}O$	2363-88-4	8.29 (26)	9.07 (34)	0.05 (48)	-				
114	640	0.620	Undecanal	$C_{11}H_{22}O$	112-44-7	14.27 (26)	17.67 (38)	-	-	6.60 (32)	0.15 (29)	17.47 (19)	0.13 (45)
115	650	0.960	2,4-Decadienal (isomer)	$C_{10}H_{16}O$	25152-84-5	-	7.08 (11)	-	0.0041 (41)	-	-	-	-
116	685	0.770	2-Undecenal	$C_{11}H_{20}O$	2463-77-6	20.50 (25)	254.25 (40)	0.21 (14)	0.03 (45)	6.46 (4)	0.05 (24)	3.92 (33)	0.08 (59)
117	720	0.650	Dodecanal	$C_{12}H_{24}O$	112-54-9	11.52 (39)	46.27 (53)	0.59 (27)	0.07 (35)	9.56 (43)	0.13 (24)	20.69 (10)	0.37 (9)
118	730	1.010	2,4-Undecadienal	$C_{11}H_{18}O$	30361-29-6	3.22 (34)	-	0.05 (44)					
119	805	0.680	Tridecanal	$C_{13}H_{26}O$	10486-19-8	3.05 (27)	4.63 (25)	-	-	1.73 (38)	0.13 (6)	4.94 (34)	0.05 (8)
120	890	0.710	Tetradecanal	$C_{14}H_{28}O$	124-25-4	-	-	-	-	2.99 (41)	0.05 (36)	4.74 (9)	0.32 (12)
			Aromatics										
121	335	1.550	Benzaldehyde	C7H6O	100-52-7	142.07 (26)	147.46 (33)	6.45 (62)	0.24 (39)	501.35 (25)	2.61 (7)	178.56 (42)	0.69 (40)
122	410	1.620	Benzeneacetaldehyde	C8H8O	122-78-1	37.91 (41)	55.01 (48)	1.05 (15)	0.05 (29)	68.70 (3)	0.63 (14)	58.70 (38)	0.85 (30)
123	410	1.860	2-Hydroxybenzaldehyde	C7H6O2	90-02-8	5.78 (11)	5.32 (18)	0.61 (34)	0.01 (7)	6.71 (8)	-	-	-
124	435	1.360	2-Methylbenzaldehyde	C8H8O	529-20-4	1.67 (45)	2.24 (56)	0.07 (49)	0.01 (13)	1.14 (8)	-	0.89 (13)	-
125	445	1.410	4-Methylbenzaldehyde	C8H8O	104-87-0	2.35 (41)	1.62 (29)	0.10 (21)	0.01 (41)	-	-	-	-
126	515	1.640	2-Phenylpropenal	C9H8O	4432-63-7	1.07 (46)	0.91 (39)	-	-	-	-	-	-
127	565	1.360	3,5-Dimethylbenzaldehyde	C9H10O	5779-95-3	4.02 (8)	12.01 (25)	0.38 (50)	0.04 (14)	7.28 (29)	0.08 (38)	1.72 (26)	-
128	585	1.140	4-(1-Methylethyl)-benzaldehyde	$C_{10}H_{12}O$	122-03-2	1.89 (33)	3.80 (44)	0.91 (12)	0.14 (32)		0.25 (6)	-	-
129	620	2.060	3-Phenyl-2-propenal	C_9H_8O	104-55-2	2.53 (25)	-	-	-	-	-	-	-
130	640	1.090	4-(t-Butyl)benzaldehyde	$C_{11}H_{14}O$	939-97-9	-	-	-	0.01 (6)	-	-	1.27 (23)	-
131	820	1.030	Lily aldehyde	$C_{14}H_{20}O$		2.16 (44)	4.89 (16)	-	0.02 (8)	3.54 (39)	0.06 (34)	5.26 (25)	0.07 (45)
					Subtotal (GC Peak Area)	704.93	1270.53	33.41	3.22	943.44	14.03	771.19	9.46
					Subtotal (%)	11.48	12.25	12.43	8.97	16.08	13.90	9.07	12.05
			Esters										
			Aliphatics										
132	95	0.440	Ethyl acetate	$C_4H_8O_2$	141-78-6	-	-	0.64 (3)	0.06 (16)	-	-	-	0.52 (32)
133	130	0.510	Ethyl propenoate	$C_5H_8O_2$	140-88-5	0.56 (55)	0.99 (33)	0.03 (32)	0.08 (38)	6.62 (18)	0.04 (2)	-	0.06 (25)
134	135	0.530	Methyl 2-methylpropenoate	$C_5H_8O_2$	80-62-6	2.70 (16)	16.01 (28)	0.80 (16)	1.50 (27)	96.22 (20)	0.97 (5)	7.16 (65)	0.27 (52)
135	165	0.460	Ethyl isobutyrate	$C_6H_{12}O_2$	97-62-1	-	-	-	-	0.56 (30)	-	0.70 (44)	0.01 (49)
136	175	0.500	Isobutyl ethanoate	$C_6H_{12}O_2$	110-19-0	2.19 (22)	0.86 (27)	0.03 (41)	-	6.95 (20)	0.07 (19)	14.73 (51)	0.14 (34)
137	195	0.520	Ethyl butanoate	$C_6H_{12}O_2$	105-54-4	0.80 (37)	-	-	-	2.75 (47)	-	3.27 (32)	-
138	205	0.550	Butyl ethanoate	$C_6H_{12}O_2$	123-86-4	1.44 (42)	2.59 (49)	0.31 (18)	0.01 (26)	0.03 (8)	-	-	-
139	235	0.510	2-Pentyl acetate	$C_7H_{14}O_2$	626-38-0			0.03 (44)					
140	255	0.550	Isoamyl ethanoate	$C_7H_{14}O_2$	123-92-2	-	7.67 (35)	0.05 (18)	-	-	-	-	-
141	255	0.730	1-Methoxy-2-propyl acetate	$C_6H_{12}O_3$	108-65-6	-	-	-	-	-	0.01 (35)	-	-
142	260	0.540	2-Methylbutyl acetate	$C_7H_{14}O_2$	624-41-9	-	-	-	-	3.49 (38)	0.03 (27)	23.60 (35)	0.12 (28)
143	315	0.640	Ethyl tiglate	$C_7H_{12}O_2$	5837-78-5	-	-	-	-	-	-	0.94 (32)	-
144	320	1.340	2-Methylamyl acetate	$C_9H_{20}O$	7789-99-3	-	-	-	-	1.12 (18)	-	-	-
145	330	0.510	Isobutyl butanoate	$C_8H_{16}O_2$	539-90-2	0.28 (28)	-	-	-	-	-	-	-
146	340	0.700	*m/z* 43, 71, 87, 59	-	-	2.51 (34)	0.80 (10)	0.02 (21)	-	-	-	-	-
147	365	0.540	Butyl butanoate	$C_8H_{16}O_2$	109-21-7	3.99 (7)	4.01 (39)	-	-	-	-	-	-

Annexes

148	365	0.620	3-Methylbutyl-2-propenoate	$C_8H_{14}O_2$	-	2.52 (38)	-	3.26 (32)	0.20 (16)	-	-	-	-
149	370	1.420	Ethylene diethanoate	$C_6H_{10}O_4$	111-55-7	-	-	-	0.14 (26)	-	-	-	-
150	370	0.560	Ethyl hexanoate	$C_8H_{16}O_2$	123-66-0	0.54 (48)	0.68 (42)	0.07 (11)	0.01 (18)	-	-	0.44 (46)	-
151	375	0.440	Propyl pivalate	$C_8H_{16}O_2$	5129-35-1	4.16 (61)	-	0.12 (30)	-	-	-	-	-
152	375	0.550	Pentyl propanate	$C_8H_{16}O_2$	624-54-4	-	1.02 (31)	0.54 (36)	0.05 (33)	-	-	-	-
153	385	1.100	Ethyl 2-methyl-3-oxopentanoate	$C_7H_{12}O_3$	17422-12-7	-	-	0.01 (10)	-	-	-	-	-
154	410	0.530	Methyl 2-ethylhexanoate	$C_9H_{18}O_2$	816-19-3	-	-	-	-	0.42 (24)	-	0.95 (30)	0.01 (28)
155	420	0.530	Isopentyl butanoate	$C_9H_{18}O_2$	106-27-4	0.55 (33)	0.26 (32)	0.04 (38)	-	-	-	-	-
156	440	0.560	t-Butyl acetoacetate	$C_8H_{14}O_3$	1694-31-1	-	-	-	-	-	-	0.35 (39)	-
157	455	0.740	2-Butoxyethyl acetate	$C_8H_{16}O_3$	112-07-2	12.75 (25)	-	0.47 (33)	-	-	-	-	-
158	460	0.550	Ethyl heptanoate	$C_9H_{18}O_2$	106-30-9	0.25 (29)	0.56 (53)	0.07 (33)	-	-	-	-	-
159	510	0.550	3-Methylheptyl acetate	$C_{10}H_{20}O_2$	72218-58-7	0.88 (47)	2.02 (47)	0.05 (21)	0.01 (24)	1.05 (9)	0.02 (36)	0.82 (56)	-
160	520	1.230	3-Methylphenyl acetate	$C_9H_{10}O_2$	122-46-3	-	-	-	-	-	0.04 (28)	-	-
161	545	0.560	Ethyl octanoate	$C_{10}H_{20}O_2$	106-32-1	-	1.36 (23)	0.18 (50)	-	-	0.0043 (37)	-	0.02 (40)
162	560	0.920	Dimethyl 2,4-dimethylpentanedioate	$C_9H_{16}O_4$	2121-68-8	-	-	-	0.03 (24)	-	0.03 (33)	-	-
163	575	0.580	2-Ethylhexyl 2-propenoate	$C_{11}H_{20}O_2$	103-11-7	0.89 (45)	-	-	0.0026 (14)	-	0.01 (39)	0.81 (30)	-
164	620	0.970	Methyl 2-phenylbutanoate	$C_{11}H_{14}O_2$	2294-71-5	-	-	-	-	-	-	0.59 (42)	-
165	630	0.560	Ethyl nonanoate	$C_{11}H_{22}O_2$	123-29-5	1.01 (40)	3.19 (57)	0.11 (64)	-	1.04 (41)	0.01 (62)	1.47 (63)	-
166	630	0.600	4-tert-Butylcyclohexyl acetate (isomer)	$C_{12}H_{22}O_2$	-	-	7.19 (35)	0.41 (17)	0.05 (27)	7.02 (23)	0.06 (39)	4.37 (31)	0.12 (40)
167	660	0.630	4-tert-Butylcyclohexyl acetate (isomer)	$C_{12}H_{22}O_2$	-	-	3.44 (45)	0.13 (43)	0.02 (40)	2.87 (16)	-	2.20 (50)	-
168	665	1.120	Methyl 2-(phenylmethyl)prop-2-enoate	$C_{11}H_{12}O_2$	3070-71-1	-	-	-	0.04 (5)	-	0.04 (79)	-	-
169	675	0.750	3-Phenyl-2-propenyl propionate	$C_{12}H_{14}O_2$	103-56-0	-	-	-	0.01 (16)	-	-	-	-
170	690	0.650	4-tert-Butylcyclohexyl acetate (isomer)	$C_{12}H_{22}O_2$	32210-23-4	6.61 (55)	8.69 (52)	0.30 (44)	0.05 (29)	2.63 (26)	-	4.47 (23)	0.29 (47)
171	695	0.920	3-hydroxy-2,4,4-trimethylpentyl 2-methyl-propanoate	$C_{12}H_{24}O3$	74367-34-3	18.20 (50)	38.91 (44)	0.88 (43)	0.23 (28)	31.74 (1)	0.37 (42)	20.00 (26)	0.18 (25)
172	710	0.580	Ethyl decanoate	$C_{12}H_{24}O_2$	110-38-3	1.29 (9)	1.22 (33)	-	-	1.13 (25)	-	2.17 (1)	-
173	725	0.860	Decyl 2-methoxyacetate	$C_{13}H_{26}O_3$	259141-02-1	-	-	-	-	-	-	6.11 (47)	-
174	760	0.970	Dibutyl-2-butenedioate	$C_{12}H_{20}O_4$	105-76-0	-	-	-	0.0042 (32)	-	-	-	-
175	760	1.080	Ethyl 2-phenylbutanoate	$C_{12}H_{16}O_2$	119-43-7	-	-	-	0.01 (17)	-	-	-	-
176	775	0.840	Diisobutyl butanedioate	$C_{12}H_{22}O_4$	925-06-4	-	-	-	0.0034 (26)	-	-	-	-
177	790	0.830	Dimethylphenethyl butyrate	$C_{14}H_{20}O_2$	10094-34-5	-	-	-	-	0.76 (20)	-	1.81 (23)	-
178	800	1.090	5-Phenyl-2-pentenoic acid ethyl ester	$C_{13}H_{16}O_2$	55282-95-6	-	-	-	-	-	0.08 (22)	-	-
179	905	0.600	Isopropyl dodecanoate	$C_{15}H_{30}O_2$	10233-13-3	-	-	-	-	-	0.07 (22)	-	-
180	960	0.550	3-Tridecanyl propionate	$C_{16}H_{32}O_2$	-	-	7.06 (30)	-	-	-	-	-	-
181	985	0.650	4-Tridecanyl propionate	$C_{16}H_{32}O_2$	-	-	-	-	-	-	0.02 (59)	-	-
182	1070	0.660	Isopropyl tetradecanoate	$C_{17}H_{34}O2$	110-27-0	5.10 (25)	11.45 (50)	-	0.04 (10)	4.83 (40)	0.03 (31)	2.71 (16)	0.07 (26)
			Aromatics										
183	460	1.210	Methyl benzoate	$C_8H_8O_2$	93-58-3	0.44 (18)	0.72 (32)	0.01 (48)	0.0050 (11)	0.88 (36)	0.01 (56)	1.21 (15)	-
184	520	1.270	Benzyl ethanoate	$C_9H_{10}O_2$	140-11-4	-	3.18 (23)	-	-	-	-	-	-
185	525	1.050	Ethyl benzoate	$C_9H_{10}O_2$	93-89-0	1.06 (16)	0.53 (24)	-	0.0046 (28)	0.66 (20)	-	0.87 (18)	-
186	545	1.050	Phenylethyl acetate	$C_{10}H_{12}O_2$	93-92-5	-	1.75 (41)	-	0.03 (28)		0.02 (7)	-	-
187	600	1.160	2-Phenylethyl acetate	$C_{10}H_{12}O_2$	103-45-7	70.90 (16)	95.21 (44)	1.63 (18)	0.0048 (44)	45.83 (20)	0.18 (24)	142.29 (23)	0.24 (10)
188	615	1.190	Methyl benzenepropanoate	$C_{10}H_{12}O_2$	103-25-3	-	-	-	0.01 (12)	-	-	-	-
189	640	1.020	Phenylpropyl acetate	$C_{11}H_{14}O_2$	10402-52-5	1.19 (41)	-	-	-	10.56 (51)	0.05 (26)	8.46 (14)	-
190	650	0.860	Dimethylphenethyl acetate	$C_{12}H_{16}O_2$	151-05-3	0.94 (18)	3.09 (37)	0.09 (46)	0.02 (8)	0.64 (59)	0.03 (6)	3.47 (23)	-
191	655	0.910	Isobutyl benzoate	$C_{11}H_{14}O_2$	120-50-3	-	0.63 (36)	-	-	-	-	-	-
192	675	0.750	3-Phenyl-2-propenyl propionate	$C_{12}H_{14}O_2$	103-56-0	-	-	-	0.01 (16)	-	-	-	-
193	695	0.970	4-Phenyl-2-butyl acetate	$C_{12}H_{16}O_2$	10415-88-0	-	-	-	-	2.40 (31)	-	1.20 (11)	-
194	710	0.940	o-Methylbenzyl acetate	$C_{10}H_{12}O_2$	17373-93-2	-	-	-	0.0031 (17)	-	-	-	-
195	730	1.040	2-Methyl-4-phenyl-butyric acid, methyl ester	$C_{12}H_{16}O_2$	-	-	36.01 (29)	3.02 (50)	0.22 (11)	30.88 (71)	0.36 (15)	39.88 (21)	-
196	790	0.820	Dimethyl benzyl carbinyl butyrate	$C_{14}H_{20}O_2$	10094-34-5	-	-	-	0.01 (15)	-	-	-	-
197	790	1.220	Methyl 5-phenylvalerate	$C_{12}H_{16}O_2$	20620-59-1	-	0.84 (56)	-	0.02 (46)	-	0.04 (10)	-	-
198	795	1.110	Ethyl-5-phenyl-2-pentenoate	$C_{13}H_{16}O_2$	55282-95-6	5.81 (39)	8.09 (46)	0.31 (9)	0.04 (9)	-	-	9.14 (59)	-
199	860	1.090	Amyl salicylate	$C_{12}H_{16}O_3$	2050-08-0	-	-	-	-	-	-	0.73 (12)	-

			Cyclics										
200	560	1.660	Cyclopropanecarboxylic acid, 2-pentyl ester	$C_9H_{16}O_2$	-	-	-	-	-	-	0.24 (19)	-	-
201	570	0.940	Cyclopropanecarboxylic acid, 2-ethylhexyl ester	$C_{12}H_{22}O_2$	103604-31-5	-	-	-	-	-	0.59 (2)	-	-
					Subtotal (GC Peak Area)	149.56	270.03	13.62	2.90	263.07	3.44	306.91	2.03
					Subtotal (%)	2.44	2.60	5.07	8.08	4.48	3.41	3.61	2.59
			Ethers										
			Aliphatics										
202	120	0.470	2-Ethoxybutane	$C_6H_{14}O$	2679-87-0	-	-	-	-	-	-	0.38 (10)	-
203	330	0.680	Vinyl (2-butoxy)ethyl ether	$C_8H_{16}O_2$	4223-11-4	120.61 (4)	99.34 (36)	-	0.19 (12)	10.41 (23)	0.26 (7)	13.79 (18)	0.01 (36)
204	435	0.530	Methoxycyclohexane	$C_7H_{14}O$	931-56-6	-	-	-	-	0.12 (36)	-	-	-
205	725	0.860	1-(Methoxymethoxy)-hexane	$C_8H_{18}O_2$	66675-06-7	-	-	-	0.01 (30)	-	-	-	-
			Aromatics										
206	390	0.910	1-Methoxy-3-methyl-benzene	$C_8H_{10}O$	100-84-5	-	1.22 (35)	-	-	0.49 (29)	-	2.14 (45)	0.03 (33)
207	445	0.860	(2-Methoxyethyl)-benzene	$C_9H_{12}O$	3558-60-9	-	0.40 (66)	-	-	-	-	0.44 (22)	0.01 (39)
208	520	1.320	1,4-Dimethoxybenzene	$C_8H_{10}O_2$	150-78-7	0.42 (53)		-	-	-	-	-	-
209	535	0.710	[(1,1-Dimethylethoxy)methyl]-benzene	$C_{11}H_{16}O$	3459-80-1	-	1.31 (59)	-	0.01 (4)	-	-	-	-
210	550	0.960	1-Methoxy-4-(2-propenyl)-benzene	$C_{10}H_{12}O$	140-67-0	-	-	-	0.01 (34)	-	-	-	-
211	715	1.270	1,1'-Oxybis-benzene	$C_{12}H_{10}O$	101-84-8	4.56 (22)	5.22 (49)	-	0.02 (15)	3.29 (59)	0.05 (25)	3.92 (28)	0.10 (32)
212	755	1.040	p-(Dimethoxymethyl)-tert-butylbenzene	$C_{13}H_{20}O_2$	-	0.52 (34)	0.84 (26)	-	0.0033 (47)	-	-	1.22 (48)	-
213	805	1.750	1-Methoxy-4-(4-methyl-4-pentenyl)-benzene	$C_{13}H_{18}O$	74672-06-3	4.84 (34)	-	-	-	-	-	-	-
					Subtotal (GC Peak Area)	130.96	108.33	-	0.25	14.30	0.32	21.89	0.15
					Subtotal (%)	2.13	1.04	-	0.70	0.24	0.32	0.26	0.19
			Furan – type compounds										
214	95	0.420	2-Methylfuran	C_5H_6O	534-22-5	-	-	-	0.0021 (17)	3.46 (22)	0.06 (18)	5.96 (20)	0.06 (22)
215	105	0.400	Tetrahydrofuran	C_4H_8O	109-99-9	2.44 (36)	2.89 (44)	-	0.08 (19)	11.01 (58)	0.12 (29)	13.37 (6)	0.26 (26)
216	130	0.460	2,5-Dimethylfuran	C_6H_8O	625-86-5	-	-	-	-	2.02 (28)	0.06 (31)	10.99 (46)	0.15 (9)
217	215	0.890	2-(Methoxymethyl)furan	$C_6H_8O_2$	13679-46-4	1.31 (40)	1.50 (47)	-	-	-	-	-	-
218	225	2.310	Furfural	$C_5H_4O_2$	98-01-1	6.25 (33)	7.84 (19)	-	-	3.84 (19)	-	3.60 (19)	-
219	265	0.510	2,5-Diethylfuran	$C_8H_{12}O$	-	1.43 (12)	0.55 (12)	-	0.0014 (47)	0.19 (31)	0.03 (21)	0.53 (45)	0.08 (27)
220	270	0.550	2-Butylfuran	$C_8H_{12}O$	4466-24-4	0.23 (48)	-	0.05 (39)	0.01 (10)	-	-	-	-
221	290	1.860	Acetylfuran	$C_6H_6O_2$	1192-62-7	-	-	-	-	0.95 (8)	-	1.53 (40)	-
222	310	0.660	2-Methyl-5-Isopropenylfuran	$C_8H_{10}O$	-	-	-	-	-	-	-	0.54 (14)	0.01 (49)
223	325	0.860	2-Methyl-5-(methylthio)furan	C_6H_8OS	13678-59-6	-	-	-	-	-	-	0.45 (27)	-
224	340	0.510	2-Butyltetrahydrofuran	$C_8H_{16}O$	1004-29-1	-	0.59 (35)	-	-	-	-	-	-
225	365	0.560	2-Pentylfuran	$C_9H_{14}O$	3777-69-3	1.20 (9)	2.77 (34)	-	-	0.57 (18)	0.04 (15)	-	-
226	370	1.220	Benzofuran	C_8H_6O	271-89-6	0.49 (54)	0.73 (22)	0.08 (43)	-	-	-	-	-
227	455	0.560	2-Hexylfuran	$C_{10}H_{16}O$	3777-70-6	-	-	-	0.0012 (26)	-	-	-	-
228	495	0.800	2-(1,1-Dimethylethyl)-4-methylfuran	$C_9H_{14}O$	6141-68-0	-	-	-	-	-	-	4.04 (30)	0.02 (40)
229	550	1.230	2-Pentanoylfuran	$C_9H_{12}O_2$	-	-	-	-	0.0033 (22)	-	-	-	-
230	570	1.480	3-Phenylfuran	$C_{10}H_8O$	13679-41-9	0.27 (41)	0.75 (20)	-		4.46 (41)	0.03 (41)	10.26 (29)	0.03 (50)
231	735	0.830	4,5-Diethyl-2,3-dihydro-2,3-dimethylfuran	$C_{10}H_{18}O$	54244-89-2	0.92 (26)	2.75 (47)	-	0.02 (25)	0.51 (37)	0.06 (15)	1.35 (38)	0.04 (49)
232	785	1.280	4-Hexyl-2,5-dihydro-2,5-dioxo-3-furanacetic acid	$C_{12}H_{16}O_5$	39212-21-0	5.11 (38)	85.69 (33)	-	0.24 (24)	952.36 (71)	19.82 (44)	1830.88 (35)	8.83 (34)
					Subtotal (GC Peak Area)	19.65	106.06	0.13	0.36	979.37	20.23	1883.50	9.48
					Subtotal (%)	0.32	1.02	0.05	1.00	16.69	20.04	22.15	12.07
			Furanones										
233	425	1.270	4-Methoxy-2,5-dimethyl-3(2H)furanone	$C_7H_{10}O_3$	4077-47-8	-	-	-	-	-	-	8.01 (10)	0.05 (33)
234	455	1.060	1-(2,4-Dimethyl-furan-3-yl)ethanone	$C_8H_{10}O_2$	32933-07-6	-	-	-	0.01 (38)	-	-	-	-
235	685	1.470	Dihydro-5-pentyl-2(3H)furanone	$C_9H_{16}O_2$	104-61-0	-	-	-	-	0.36 (19)	0.01 (13)	-	-

236	770	1.520	5-Hexyldihydro-2(3H)furanone	$C_{10}H_{18}O_2$	706-14-9	-	-	-	0.01 (25)	-	-	3.73 (7)	0.15 (42)
					Subtotal (GC Peak Area)	-	-	-	0.01	0.36	0.01	11.74	0.20
					Subtotal (%)	-	-	-	0.03	0.01	0.01	0.14	0.25
			Halogenated compounds										
237	130	0.900	Bromodichloromethane	$CHBrCl_2$	75-27-4	-	0.41 (12)	0.02 (34)	0.01 (8)	-	-	-	0.02 (7)
238	185	0.590	5-Chloro-2-pentanone	C_5H_9ClO	5891-21-4	-	0.42 (41)	-	-	-	0.01 (11)	-	0.01 (33)
239	200	0.480	Tetrachloroethylene	C_2Cl_4	127-18-4	-	-	-	-	1.71 (21)	-	1.71 (27)	0.04 (7)
240	230	0.780	Chlorobenzene	C_6H_5Cl	108-90-7	2.80 (26)	3.29 (24)	0.43 (29)	-	0.22 (23)	-	-	-
241	325	0.610	1-Bromo-2-propanone	C_3H_5BrO	598-31-2	0.55 (44)	-	-	-	-	-	-	-
242	380	0.960	1,4-Dichlorobenzene	$C_6H_4Cl_2$	106-46-7	-	1.06 (18)	-	0.01 (46)	-	-	-	-
243	380	1.200	Benzyl chloride	C_7H_7Cl	100-44-7	1.10 (31)	2.54 (18)	-	-	52.83 (10)	0.50 (21)	2.96 (50)	0.02 (38)
244	400	0.390	3-Chloro-1,1,2,2-tetramethyl-cyclopropane	$C_7H_{13}Cl$	14123-41-2	-	2.62 (39)	-	-	-	-	-	-
245	425	0.520	Octyl chloride	$C_8H_{17}Cl$	111-85-3	0.27 (12)	-	-	-	-	0.01 (31)	0.85 (42)	-
246	450	0.400	3-Trifluoroacetoxy-6-ethyldecane	$C_{14}H_{25}F_3O_2$	-	1.20 (17)	1.47 (43)	0.27 (15)	-	1.92 (22)	-	4.12 (9)	-
247	450	0.500	N-(2-Fluorophenyl)octanamide	$C_{14}H_{20}FNO$	-	0.13(52)	-	-	0.0012 (22)	-	-	-	-
248	480	1.150	(2-Chloroethenyl)-benzene	C_8H_7Cl	622-25-3	0.59 (22)	1.43 (28)	-	-	0.30 (48)	-	-	-
249	520	1.530	3,4,5-Trichloropyridine	$C_5H_2Cl_3N$	33216-52-3	-	3.25 (39)	-	-	-	-	-	-
250	555	0.710	5,7,7-Trichloro-6-hepten-2-one	$C_7H_9Cl_3O$	-	-	-	-	-	-	0.03 (16)	-	0.02 (54)
251	565	0.740	1-Bromo-3,3-dimethyl-2-butanone	$C_6H_{11}BrO$	5469-26-1	0.74 (40)	0.89 (45)	-	-	-	0.01 (11)	0.63 (48)	-
252	600	1.310	2,4-Dichlorobenzaldehyde	$C_7H_4Cl_2O$	874-42-0	-	1.54 (27)	-	0.01 (43)	-	-	-	-
253	640	0.680	2-Bromo-2-methylbutane	$C_5H_{11}Br$	507-36-8	1.34 (8)	-	0.10 (27)	0.01 (21)	0.64 (43)	-	-	-
254	650	0.570	1-Fluoro-decane	$C_{10}H_{21}F$	334-56-5	-	1.29 (21)	-	-		-	-	-
255	675	0.420	3-Chloro-1,1,2,2-tetramethylcyclopropane	$C_7H_{13}Cl$	14123-41-2	-	3.76 (48)	-	-	2.20 (36)	-	2.17 (31)	0.08 (41)
256	740	1.190	3-Benzyl-4-bromo-1,2,3-triazole 1-oxide	$C_9H_8BrN_3O$	-	-	-	-	0.02 (17)	-	-	-	-
257	770	0.570	1-Chloroundecane	$C_{11}H_{23}Cl$	2473-03-2	-	-	-	0.02 (19)	0.51 (10)	0.01 (24)	-	-
258	780	1.130	(2-Chloro-2,3-dimethylcyclopropyl)benzene	$C_{11}H_{13}Cl$	-	-	14.90 (68)	-	-	-	0.25 (5)	-	-
259	855	1.340	2-Bromoisobutyrophenone	$C_{10}H_{11}BrO$	10409-54-8	-	1.28 (29)	-	-	-	-	-	-
					Subtotal (GC Peak Area)	8.72	40.14	0.81	0.08	60.33	0.81	12.46	0.19
					Subtotal (%)	0.14	0.39	0.30	0.22	1.03	0.80	0.15	0.24
			Hydrocarbons										
			Aliphatics										
260	130	0.340	Heptane	C_7H_{16}	142-82-5	0.67 (28)	2.86 (37)	0.18 (37)	0.01 (29)	2.40 (36)	-	3.42 (45)	0.02 (64)
261	190	0.360	2,4-Dimethylheptane	C_9H_{20}	2213-23-2	1.41 (29)	2.51 (47)	0.18 (36)	0.02 (31)	1.16 (35)	0.02 (45)	-	0.05 (31)
262	215	0.420	1,3-Octadiene	C_8H_{14}	1002-33-1	10.35 (22)	24.34 (25)	0.85 (38)	0.04 (23)	-	-	-	-
263	275	0.370	Nonane	C_9H_{20}	111-84-2	-	-	-	0.01 (23)	0.99 (26)	-	1.31 (35)	0.03 (41)
264	365	0.370	C10 (*m/z* 57, 41, 39, 55)	-	-	1.08 (12)	-	0.27 (41)	0.07 (65)	2.18 (33)	-	0.83 (29)	0.11 (10)
265	370	0.380	Decane	$C_{10}H_{22}$	124-18-5	-	-	-	-	-	0.04 (30)	-	-
266	375	0.380	C10 (*m/z* 57, 97, 41, 55)	$C_{12}H_{24}$	123-48-8	3.07 (34)	2.38 (53)	0.33 (10)	-	3.91 (18)	-	6.23 (48)	0.22 (12)
267	400	0.390	3,4,4-Trimethyl-2-pentene	C_8H_{16}	598-96-9	-	-	-	0.04 (38)	-	-	1.00 (47)	-
268	410	0.390	2,3,4-Trimethyl-2-pentene	C_8H_{16}	565-77-5	-	-	-	0.03 (48)	1.07 (19)	-	-	-
269	410	0.710	Nonatetra-1,3,5,7-ene	C_9H_{12}	83829-35-0	2.95 (23)	-	-	-	-	-	-	-
270	420	0.400	C10 (*m/z* 57, 41, 55, 69)	$C_{12}H_{24}$	7756-94-7	-	4.09 (38)	0.33 (48)	0.06 (39)	5.16 (38)	-	4.14 (41)	0.17 (38)
271	420	0.740	C10 (*m/z* 41, 69, 39, 67)	C_8H_{14}	998-94-7	-	-	0.14 (36)	0.01 (44)	0.55 (4)	-	-	-
272	425	1.060	C10 (*m/z* 41, 70, 39, 43)	C_8H_{16}	16106-59-5	-	-	-	-	-	-	-	0.03 (14)
273	465	0.390	Undecane	$C_{11}H_{24}$	1120-21-4	-	-	-	-	-	0.05 (10)	-	0.05 (46)
274	465	0.650	1,9-Dodecadiene	$C_{12}H_{22}$	-	-	-	1.45 (43)	-	-	-	-	-
275	520	0.890	7-Methyl-2-decene	$C_{11}H_{22}$	74630-23-2	-	-	-	0.10 (38)	-	-	-	-
276	540	0.910	C12 (*m/z* 41, 55, 69, 43)	$C_{14}H_{28}$	-	23.85 (46)	-	-	0.59 (13)	-	-	-	-
277	550	0.400	Dodecane	$C_{12}H_{26}$	112-40-3	-	12.62 (53)	0.25 (42)	0.03 (13)	-	-	11.99 (33)	0.08 (22)
278	635	0.410	Tridecane	$C_{13}H_{28}$	629-50-5	-	6.37 (51)	0.17 (41)	0.01 (35)	8.71 (25)	0.67 (26)	6.98 (43)	0.10 (28)
279	715	0.420	Tetradecane	$C_{14}H_{30}$	629-59-4	-	6.05 (47)	0.23 (13)	0.04 (36)	-	0.15 (16)	4.26 (3)	0.08 (48)
280	765	0.420	C14(*m/z* 43, 57, 41, 71)	$C_{15}H_{32}$	1560-95-8	-	-	-	0.03 (34)	-	0.07 (1)	-	-
281	790	0.470	1-Pentadecene	$C_{15}H_{30}$	13360-61-7	-	-	-	-	-	0.08 (37)	-	-
282	795	0.450	Pentadecane	$C_{15}H_{32}$	629-62-9	-	7.76 (50)	0.10 (26)	0.03 (44)	-	0.13 (21)	2.85 (25)	0.05 (31)
283	880	0.490	Hexadecane	$C_{16}H_{34}$	544-76-3	2.16 (26)	4.17 (36)	0.07 (16)	0.05 (27)	1.02 (20)	0.16 (20)	1.62 (9)	0.04 (29)
284	965	0.430	Heptadecane	$C_{17}H_{36}$	629-78-7	4.06 (38)	6.63 (41)	0.11 (43)	0.04 (6)	0.74 (24)	0.11 (21)	7.69 (42)	0.05 (41)

285	1010	0.440	Octadecane	$C_{18}H_{38}$	593-45-3	1.34 (37)	8.05 (46)	-	-	-	-	0.91 (19)	-
286	1150	0.450	Nonadecane	$C_{19}H_{40}$	629-92-5	-	2.91 (33)	-	-	-	-	-	-
			Aromatics										
287	115	0.460	Benzene	C_6H_6	71-43-2	2.73 (29)	3.05 (28)	0.19 (18)	0.01 (23)	2.62 (24)	0.04 (9)	5.21 (29)	0.07 (36)
288	170	0.540	Toluene	C_7H_8	108-88-3	8.87 (19)	39.47 (43)	0.58 (15)	1.07 (48)	29.73 (6)	0.56 (9)	50.26 (24)	1.21 (32)
289	240	0.580	Ethylbenzene	C_8H_{10}	100-41-4	1.04 (37)	1.23 (37)	0.06 (12)	0.09 (4)	19.63 (22)	-	20.37 (14)	0.32 (27)
290	250	0.590	1,3-Dimethylbenzene	C_8H_{10}	108-38-3	5.84 (29)	7.45 (29)	0.39 (9)	0.03 (7)	46.69 (24)	0.09 (45)	43.81 (11)	0.70 (25)
291	270	0.640	1,2-Dimethylbenzene	C_8H_{10}	95-47-6	2.28 (46)	4.12 (22)	0.16 (4)	0.03 (10)	2.87 (17)	0.01 (52)	3.41 (47)	0.05 (21)
292	300	0.570	(1-Methylethyl)benzene	C_9H_{12}	98-82-8	-	0.25 (18)	-	0.01 (7)	2.60 (2)	0.0041 (22)	2.09 (19)	0.05 (13)
293	320	0.690	2-Propenylbenzene	C_9H_{10}	300-57-2	-	-	0.01 (36)	0.02 (28)	3.69 (10)	0.01 (28)	3.10 (14)	0.06 (19)
294	325	0.580	Propylbenzene	C_9H_{12}	103-65-1	0.32 (26)	0.95 (14)	0.01 (11)	0.03 (12)	5.66 (10)	0.01 (18)	5.10 (13)	0.11 (12)
295	330	0.470	*m/z* 91 106 77 119	-	-	-	-	0.02 (10)	0.0037 (33)	-	-	0.69 (53)	0.03 (6)
296	335	0.590	1-Ethyl-4-methylbenzene	C_9H_{12}	622-96-8	0.84 (47)	5.55 (51)	0.04 (18)	0.03 (43)	3.93 (43)	0.02 (24)	5.38 (37)	0.10 (19)
297	350	0.630	1-Ethyl-2-methylbenzene	C_9H_{12}	611-14-3	0.21 (13)	1.45 (21)	-	0.01 (6)	0.99 (61)	0.01 (38)	0.43 (17)	0.01 (8)
298	355	0.750	(1-Methylethenyl)benzene	C_9H_{10}	98-83-9	-	0.68 (43)	-	-	-	-	-	-
299	365	0.640	1,3,5-Trimethylbenzene	C_9H_{12}	108-67-8	3.23 (37)	24.93 (23)	0.27 (25)	0.11 (3)	2.87 (30)	0.12 (42)	10.27 (40)	0.30 (36)
300	370	0.770	1-Propenylbenzene	C_9H_{10}	637-50-3	-	0.60 (19)	-	-	-	-	-	
301	390	0.580	1-Methyl-2-(1-methylethyl)benzene	$C_{10}H_{14}$	527-84-4	0.95 (25)	1.27 (54)	0.03 (9)	0.02 (39)	0.58 (32)	0.04 (3)	0.67 (43)	0.02 (16)
302	390	0.690	1,2,3-Trimethylbenzene	C_9H_{12}	526-73-8	0.79 (64)	5.02 (47)	0.05 (37)	0.05 (10)	2.87 (30)	0.05 (3)	19.33 (7)	0.01 (19)
303	420	0.590	1-Methyl-3-propylbenzene	$C_{10}H_{14}$	1074-43-7	-	-	-	0.01 (48)	-	0.01 (23)	-	-
304	425	0.610	2-Ethyl-1,4-dimethylbenzene	$C_{10}H_{14}$	1758-88-9	0.24 (39)	0.76 (16)	0.01 (12)	0.0049 (49)	0.69 (43)	0.01 (13)	0.53 (48)	0.01 (46)
305	440	0.630	1-Ethyl-2,3-dimethylbenzene	$C_{10}H_{14}$	933-98-2	-	1.76 (24)	-	0.02 (5)	0.46 (37)	-	0.46 (39)	0.01 (23)
306	450	0.740	o-Isopropenyltoluene	$C_{10}H_{12}$	7399-49-7	2.47 (42)	-	-	-	2.18 (33)	-	5.39 (34)	0.06 (43)
307	470	0.660	2-Ethyl-1,3-dimethylbenzene	$C_{10}H_{14}$	1758-88-9	-	-	-	0.01 (42)	-	0.01 (16)	-	-
308	505	0.750	1-Phenyl-1-butene	$C_{10}H_{12}$	1005-64-7	-	2.29 (39)	-	0.01 (43)	2.58 (31)	-	-	-
309	480	0.670	1,2,4,5-Tetramethylbenzene	$C_{10}H_{14}$	95-93-2	2.40 (17)	5.29 (15)	-	0.05 (4)	0.71 (25)	0.05 (16)	7.68 (48)	-
310	485	0.970	Diethenylbenzene	$C_{10}H_{10}$	1321-74-0	0.39 (23)	0.74 (23)	-	0.0028 (35)	0.42 (27)	-	0.53 (36)	0.01 (13)
311	550	0.650	2,4-Diethyl-1-methylbenzene	$C_{11}H_{16}$	1758-85-6	-	-	-	0.0023 (6)	-	-	-	-
312	595	0.500	*m/z* 57, 175, 41, 91	-	-	0.62 (24)	1.24 (28)	0.03 (25)	0.01 (20)	0.31 (46)	0.01 (26)	0.57 (7)	-
313	620	0.740	Pentamethylbenzene	$C_{11}H_{16}$	700-12-9	-	-	-	0.0022 (36)	-	-	-	-
314	700	1.270	Biphenyl	$C_{12}H_{10}$	92-52-4	1.61 (47)	2.84 (21)	0.08 (22)	0.01 (28)	1.16 (47)	0.02 (15)	1.73 (28)	0.03 (48)
315	815	1.260	Bibenzyl	$C_{14}H_{14}$	103-29-7	0.71 (20)	1.16 (24)	0.03 (33)	-	-	-	-	-
316	880	1.020	2-Methyl-6-phenyl-1,6-heptadiene	$C_{14}H_{18}$	51708-97-5	3.58 (35)	7.57 (40)	0.14 (33)	0.04 (13)	1.36 (37)	0.06 (37)	5.31 (47)	0.03 (51)
317	910	0.590	(1,1-Diethylpropyl)benzene	$C_{13}H_{20}$	4170-84-7	0.47 (40)	0.70 (31)	-	-	0.10 (43)	0.02 (43)	-	-
318	920	0.590	(1-Propylnonyl)benzene	$C_{18}H_{30}$	2719-64-4	0.31 (37)	-	-	-	-	0.01 (49)	-	-
319	925	1.270	1,1'-(1,3-Propanediyl)bis-benzene	$C_{15}H_{16}$	1081-75-0	1.57 (15)	2.06 (15)	-	0.01 (23)	1.22 (29)	-	1.81 (15)	0.03 (20)
320	990	0.590	(1-Propylheptyl)benzene	$C_{16}H_{26}$	4537-12-6	1.21 (35)	1.16 (45)	-	-	0.42 (23)	0.01 (15)	-	-
321	1005	0.610	(1-Pentylhexyl)benzene	$C_{17}H_{28}$	4537-14-8	0.33 (30)	0.69 (49)	-	-	-	-	-	-
322	1140	0.770	(1-Methylnonyl)benzene	$C_{16}H_{26}$	4537-13-7	-	-	-	-	-	0.01 (17)	-	-
			Cyclics										
323	100	0.340	Methylcyclopentane	C_6H_{12}	96-37-7	-	-	-	-	1.98 (24)	-	-	-
324	215	0.730	Dicyclopropylmethane	C_7H_{12}	5685-47-2	-		0.05 (16)	-	-	-	-	-
325	235	0.430	1-Ethylcyclohexene	C_8H_{14}	1453-24-3	-	1.98 (37)	0.07 (21)	-	-	-	-	-
326	270	0.530	1,2-Dimethyl-1,4-cyclohexadiene	C_8H_{12}	17351-28-9	-	-	-	-	-	0.03 (8)	-	-
327	270	0.790	1,3,5,7-Cyclooctatetraene	C_8H_8	629-20-9	-	-	-	1.80 (14)	117.50 (29)	-	-	-
328	305	0.490	1,2-Propadienylcyclohexane	C_9H_{14}	5664-17-5	1.12 (28)	1.62 (45)	0.09 (10)	0.05 (44)	-	0.02 (10)	1.36 (16)	-
329	350	0.540	1-Ethyl-1,4-cyclohexadiene	C_8H_{12}	19841-74-8	5.67 (47)	-	-	-	2.19 (22)	0.02 (1)	-	-
330	495	1.030	1-Cyclohexylheptene	$C_{13}H_{24}$	114614-83-4	-	-	-	-	-	-	-	0.13 (14)
331	515	0.810	1-Ethyl-2-methylcyclohexane	C_9H_{18}	4923-78-8	-	-	-	-	83.20 (33)	1.40 (25)	88.58 (35)	1.23 (24)
332	695	0.670	(2-Ethyl-1-methyl-1-butenyl)cyclohexane	$C_{13}H_{24}$	74810-42-7	-	-	-	-	-	0.04 (16)	-	-
333	755	0.460	Isobutylcyclopentane	C_9H_{18}	3788-32-7	-	-	-	-	-	0.03 (23)	-	-
334	840	0.480	Nonylcyclohexane	$C_{15}H_{30}$	2883-02-5	-	-	-	-	-	0.02 (45)	-	-
					Subtotal (GC Peak Area)	100.74	218.60	6.94	4.60	369.10	4.22	337.29	5.65
					Subtotal (%)	1.64	2.11	2.58	12.80	6.29	4.18	3.97	7.20

Annexes

			Ketones										
			Aliphatics										
335	75	0.390	2-Propanone (*m/z* 43, 58, 42, 39)	C_3H_6O	67-64-1	23.79 (18)	40.52 (40)	0.94 (16)	0.09 (39)	27.44 (10)	0.67 (8)	24.48 (27)	0.38 (42)
336	90	0.440	2-Butanone	C_4H_8O	78-93-3	-	-	-	0.03 (48)	27.71 (10)	0.46 (22)	9.91 (23)	-
337	90	0.480	3-Buten-2-one	C_4H_6O	78-94-4	-	-	0.08 (54)	-	-	-	-	-
338	90	0.560	2,3-Butanedione	$C_4H_6O_2$	431-03-8	3.29 (22)	2.85 (57)	0.24 (8)	0.03 (35)	7.02 (21)	0.07 (23)	-	-
339	125	0.500	2-Pentanone	$C_5H_{10}O$	107-87-9	-	-	-	-	2.36 (27)	0.01 (19)	4.68 (10)	-
340	125	0.650	2,3-Pentanedione	$C_5H_8O_2$	600-14-6	1.64 (19)	1.58 (27)	0.13 (48)	0.03 (14)	2.18 (21)	-	-	-
341	130	0.500	3-Pentanone	$C_5H_{10}O$	96-22-0	-	-	-	-	-	0.03 (8)	-	-
342	140	1.540	3-Hydroxy-2-butanone	$C_4H_8O_2$	513-86-0	-	-	-	-	-	-	1.42 (40)	-
343	150	0.770	3-Penten-2-one	C_5H_8O	3102-33-8	-	-	-	-	0.95 (45)	-	-	-
344	160	0.510	3-Methyl-2-pentanone	$C_6H_{12}O$	565-61-7	-	-	-	-	0.38 (18)	-	0.80 (61)	-
345	190	0.690	4-Methyl-3-penten-2-one	$C_6H_{10}O$	141-79-7	-	-	-	-	0.67 (28)	-	-	-
346	240	0.610	5-Methyl-2-hexanone	$C_7H_{14}O$	110-12-3	-	-	-	-	0.64 (16)	-	0.78 (19)	-
347	250	0.570	4-Heptanone	$C_7H_{14}O$	123-19-3	-	-	-	-	-	0.04 (47)	6.04 (19)	0.01 (35)
348	265	0.580	3-Heptanone	$C_7H_{14}O$	106-35-4	0.70 (30)	0.60 (38)	0.04 (19)	0.01 (22)	0.33 (41)	0.01 (7)	0.33 (11)	0.01 (41)
349	270	0.620	2-Heptanone	$C_7H_{14}O$	110-43-0	2.96 (36)	-	-	-	6.48 (39)	0.06 (18)	5.99 (44)	0.03 (48)
350	275	0.740	5-Hepten-2-one	$C_7H_{12}O$	6714-00-7	1.14 (68)	2.28 (26)	0.15 (20)	-	-	-	-	-
351	350	0.690	1-Octen-3-one	$C_8H_{14}O$	4312-99-6	148.92 (14)	351.05 (38)	15.55 (30)	0.34 (33)	1.02 (11)	0.02 (25)	0.88 (12)	-
352	355	0.710	2,3-Octanedione	$C_8H_{14}O_2$	585-25-1	-	-	-	0.08 (43)	-	0.01 (48)	-	-
353	355	0.740	6-Methyl-5-hepten-2-one	$C_8H_{14}O$	110-93-0	46.66 (23)	30.33 (35)	2.87 (23)	0.29 (1)	14.82 (40)	0.31 (15)	13.13 (41)	0.20 (32)
354	360	0.600	3-Octanone	$C_8H_{16}O$	106-68-3	150.16 (5)	-	22.49 (50)	1.66 (10)	7.99 (27)	0.11 (8)	-	-
355	360	0.640	2-Octanone	$C_8H_{16}O$	111-13-7	-	-	-	-	-	-	0.53 (27)	0.02 (43)
356	405	0.780	3-Octen-2-one	$C_8H_{14}O$	1669-44-9	1.52 (30)	-	-	0.01 (15)	-	-	-	0.01 (45)
357	415	0.640	5-Ethyl-2-heptanone	$C_9H_{18}O$	-	-	1.25 (35)	-	0.01 (27)	0.76 (43)	-	1.14 (37)	-
358	435	0.680	3-Nonen-2-one	$C_9H_{16}O$	14309-57-0	0.67 (12)	1.29 (24)	0.05 (22)	0.0034 (19)	35.12 (32)	2.19 (10)	137.01 (19)	-
359	450	0.610	3-Nonanone	$C_9H_{18}O$	925-78-0	0.32 (38)	-	-	0.0030 (43)	-	-	-	-
360	455	0.640	2-Nonanone	$C_9H_{18}O$	821-55-6	13.45 (50)	21.80 (19)	0.77 (27)	0.17 (32)	165.28 (11)	2.82 (5)	117.89 (34)	-
361	460	0.730	5-Nonen-2-one	$C_9H_{16}O$	27039-84-5	-	-	-	-	11.18 (21)	0.55 (17)	40.26 (33)	0.30 (36)
362	470	1.040	6-Methyl-3,5-heptadiene-2-one	$C_8H_{12}O$	1604-28-0	0.49 (43)	1.13 (31)	-	0.0033 (38)	-	-	-	-
363	490	0.630	*m/z* 43, 58, 71, 41		-	-	-	-	-	-	-	-	0.04 (43)
364	495	0.760	3-Nonen-2-one (isomer)	$C_9H_{16}O$	18402-83-0	0.79 (41)	0.92 (23)	0.06 (22)	0.01 (31)	0.57 (2)	0.02 (38)	1.75 (36)	0.02 (37)
365	530	0.690	1-Decen-3-one	$C_{10}H_{18}O$	-	-	-	-	0.01 (19)	-	0.08 (42)	-	-
366	540	0.600	3-Decanone	$C_{10}H_{20}O$	928-80-3	-	-	-	-	5.62 (40)	0.05 (24)	3.39 (45)	0.04 (45)
367	545	0.640	2-Decanone	$C_{10}H_{20}O$	693-54-9	5.17 (33)	25.21 (52)	0.97 (16)	-	-	0.21 (10)	26.00 (35)	-
368	570	1.760	3,8-Nonadien-2-one	$C_9H_{14}O$	55282-90-1	-	-	-	-	32.03 (23)	-	-	-
369	585	0.750	3-Decen-2-one	$C_{10}H_{18}O$	10519-33-2	-	-	-	0.0024 (37)	-	-	-	-
370	610	0.580	*m/z* 43, 58, 71, 72		-	-	0.93 (40)	-	0.0045 (47)	6.80 (47)	0.04 (22)	1.99 (25)	0.02 (40)
371	610	0.750	4,8-Dimethyl-nona-3,8-dien-2-one	$C_{11}H_{18}O$	-	-	-	-	0.01 (41)	-	-	-	-
372	620	0.680	3-Undecen-2-one	$C_{11}H_{20}O$	-	-	-	-	-	-	0.03 (17)	-	-
373	625	0.620	3-Undecanone	$C_{11}H_{22}O$	2216-87-7	-	0.76 (48)	-	-	-	-	-	-
374	625	0.720	5-Ethyl-4-methyl-5-hepten-3-one	$C_{10}H_{18}O$	74764-56-0	5.63 (37)	3.55 (17)	-	-	-	-	-	-
375	630	0.640	2-Undecanone	$C_{11}H_{22}O$	112-12-9	3.84 (1)	7.00 (34)	0.30 (15)	-	-	0.04 (7)	2.10 (32)	-
376	650	0.560	5-Dodecanone	$C_{12}H_{24}O$	19780-10-0	-	-	-	-	5.44 (43)	0.03 (3)	3.39 (15)	0.02 (45)
377	670	0.630	*m/z* 43, 58, 57, 71		-	-	0.43 (32)	-	-	-	-	-	-
378	690	0.600	2-Methyl-5-undecanone	$C_{12}H_{24}O$	50639-02-6	-	-	-	-	-	-	1.82 (36)	-
379	695	0.820	Tridecane-2,4-dione	$C_{13}H_{24}O_2$	25276-80-6	-	-	-	-	-	-	7.10 (43)	-
380	710	0.680	2-Dodecanone	$C_{12}H_{24}O$	6175-49-1	2.73 (3)	2.74 (29)	0.08 (37)	0.01 (31)	-	0.02 (9)	-	0.02 (28)
381	735	0.590	6-Tridecanone	$C_{13}H_{26}O$	22026-12-6	-	-	-	-	1.89 (41)	-	-	-
382	755	0.800	6,10-Dimethyl-,5,9-undecadien-2-one	$C_{13}H_{22}O$	3796-70-1	44.58 (52)	55.35 (29)	2.50 (23)	0.25 (9)	10.78 (43)	0.55 (45)	37.99 (48)	0.16 (19)
383	770	0.630	*m/z* 58, 43, 71, 57		-	-	-	-	-	-	-	0.91 (21)	-
384	790	0.690	2-Tridecanone	$C_{13}H_{26}O$	593-08-8	2.73 (3)	-	-	0.01 (38)	1.67 (37)	0.03 (14)	1.12 (14)	0.03 (45)
			Aromatics										
385	435	1.500	Acetophenone	C_8H_8O	98-86-2	11.36 (38)	13.44 (32)	0.59 (21)	0.10 (33)	29.44 (23)	0.20 (37)	18.60 (8)	-
386	490	1.460	1-Phenyl- 2-propanone	$C_9H_{10}O$	103-79-7	-	-	-	0.01 (8)	-	-	-	-
387	530	1.320	1-(4-Methylphenyl)-ethanone	$C_9H_{10}O$	122-00-9	1.33 (58)	1.30 (26)	-	0.03 (33)	-	0.04 (7)	-	-
388	595	1.140	1-Phenyl-1-butanone	$C_{10}H_{12}O$	495-40-9	-	-	-	0.0023 (19)	-	-	-	-

389	650	1.070	4-Isopropylacetophenone	$C_{11}H_{14}O$	645-13-6	-	3.33 (38)	-	0.03 (7)	-	0.05 (17)	-	-
390	910	2.210	Benzophenone	$C_{13}H_{10}O$	119-61-9	1.00 (33)	2.41 (25)	-	0.01 (13)	2.16 (41)	0.04 (6)	3.01 (7)	0.04 (37)
			Cyclics										
391	270	0.890	Cyclohexanone	$C_6H_{10}O$	108-94-1	-	-	-	0.0046 (32)	-	0.01 (17)	-	0.02 (37)
392	295	3.000	Butyrolactone	$C_4H_6O_2$	96-48-0	-	-	-	-	4.72 (34)	-	-	-
393	305	0.740	1-Cyclopentylethanone	$C_7H_{12}O$	6004-60-0	-	-	-	-	0.49 (9)	-	-	-
394	310	0.760	2-Ethylcyclopentanone	$C_7H_{12}O$	4971-18-0	-	-	-	-	0.43 (0)	-	-	-
395	405	0.750	Cyclooctanone	$C_8H_{14}O$	502-49-8	-	-	-	-	1.39 (3)	0.02 (4)	-	-
396	495	0.740	2-n-Hexylcyclopentanone	$C_{11}H_{20}O$	13074-65-2	-	-	-	0.0031 (7)	-	-	-	-
397	515	0.790	3-Butylcyclopentanone	$C_9H_{16}O$	57283-81-5	-	-	-	-	13.18 (46)	0.54 (34)	17.74 (33)	0.23 (40)
398	525	0.840	2-Ethylcycloheptanone	$C_9H_{16}O$	3183-41-3	-	-	-	-	164.13 (28)	-	218.53 (16)	2.08 (16)
399	565	0.870	Cyclononanone	$C_9H_{16}O$	3350-30-9	-	-	-	-	15.30 (44)	0.70 (21)	34.08 (13)	0.35 (30)
400	605	0.780	3,3,5-Trimethylcyclohexanone	$C_9H_{16}O$	873-94-9	-	-	-	0.01 (36)	-	-	-	-
					Subtotal (GC Peak Area)	474.87	572.06	47.80	3.24	608.38	10.04	744.83	4.01
					Subtotal (%)	7.73	5.51	17.79	9.02	10.37	9.94	8.76	5.11
			N-compounds										
401	145	0.770	2-Nitropropane	$C_3H_7NO_2$	79-46-9	-	-	-	-	-	0.05 (13)	2.44 (43)	-
402	170	0.880	Pyridine	C_5H_5N	110-86-1	-	-	-	0.01 (19)	-	-	-	-
403	195	0.970	2-Methylpyridine	C_6H_7N	109-06-8	-	-	-	-	0.63 (41)	-	-	-
404	360	1.860	Benzonitrile	C_7H_5N	100-47-0	1.09 (31)	2.50 (15)	0.05 (26)	0.01 (45)	4.24 (14)	0.04 (12)	2.24 (21)	-
405	405	0.500	Cyclobutylamine	C_4H_9N	2516-34-9	0.88 (23)	-	0.16 (19)	0.02 (17)	-	-	-	0.01 (57)
406	405	1.220	6-Nitro-2-hexene	$C_6H_{11}NO_2$	40244-96-0	-	-	0.10 (58)	0.04 (31)	-	-	-	-
407	415	0.990	1-Nitrohexane	$C_6H_{13}NO_2$	646-14-0	8.80 (15)	4.36 (52)	0.11 (22)	0.04 (8)	-	0.03 (26)	-	-
408	445	0.710	3-Nitro-1-butene	$C_4H_7NO_2$	-	0.55 (31)	-	-	-	-	-	-	-
409	490	1.470	N-Ethyl-benzenamine	$C_8H_{11}N$	103-69-5	-	0.58 (15)	-	-	-	-	-	-
410	640	0.900	N,N-Dibutyl-formamide	$C_9H_{19}NO$	761-65-9	-	2.31 (14)	0.03 (49)	0.01 (29)	-	0.01 (12)	-	-
411	655	0.700	2,5-Dimethyl-4-nitro-3-hexanone	$C_8H_{15}NO_3$	59906-54-6	-	-	-	0.01 (13)	-	0.04 (41)	-	-
412	955	0.810	2,4-di-t-butyl-6-nitro-phenol	$C_{14}H_{21}NO_3$	-	1.19 (48)	4.16 (51)	0.08 (2)	0.01 (47)	1.23 (36)	0.02 (12)	-	0.07 (25)
413	1015	1.120	3-(4'-nitrophenyl)pentan-3-ol	$C_{11}H_{15}NO_3$	-	2.82 (20)	-	0.40 (8)	-	-	-	-	-
					Subtotal (GC Peak Area)	15.33	13.91	0.94	0.14	6.10	0.19	4.68	0.08
					Subtotal (%)	0.25	0.13	0.35	0.39	0.10	0.19	0.06	0.10
			Pyrazines										
414	155	1.040	Pyrazine	$C_4H_4N_2$	290-37-9	2.48 (22)	5.33 (50)	0.24 (13)	0.05 (27)	11.30 (46)	0.78 (16)	15.59 (38)	0.35 (10)
415	215	1.000	2-Methylpyrazine	$C_5H_6N_2$	109-08-0	2.53 (26)	2.74 (38)	0.07 (6)	-	23.44 (12)	0.36 (1)	31.44 (17)	0.39 (12)
416	290	0.900	2,5-Dimethylpyrazine	$C_6H_8N_2$	123-32-0	2.90 (33)	5.02 (68)	0.07 (14)	0.02 (45)	144.64 (10)	1.62 (13)	178.78 (11)	1.55 (19)
417	295	0.920	Ethylpyrazine	$C_6H_8N_2$	13925-00-3	-	-	-	-	2.10 (12)	-	3.07 (33)	-
418	295	0.970	2,3-Dimethylpyrazine	$C_6H_8N_2$	5910-89-4	-	-	-	0.003 (26)	1.81 (13)	-	2.27 (19)	0.02 (32)
419	345	0.820	2-Isopropylpyrazine	$C_7H_{10}N_2$	29460-90-0	-	-	-	-	0.620 (14)	-	0.49 (16)	-
420	370	0.840	2-Ethyl-5-methylpyrazine	$C_7H_{10}N_2$	13360-64-0	-	-	-	-	37.46 (19)	1.17 (29)	36.88 (10)	0.23 (16)
421	370	0.870	Trimethylpyrazine	$C_7H_{10}N_2$	14667-55-1	1.42 (21)	1.03 (48)	-	-	39.34 (7)	0.28 (33)	26.88 (16)	0.26 (24)
422	390	1.080	2-Ethenyl-5-methylpyrazine	$C_7H_8N_2$	13925-08-1	-	-	-	-	1.34 (47)	-	2.22 (10)	-
423	420	0.750	2-Methyl-3-isopropylpyrazine	$C_8H_{12}N_2$	15986-81-9	-	-	-	-	10.29 (15)	0.06 (29)	8.18 (7)	0.05 (20)
424	440	0.760	3-Ethyl-2,5-dimethylpyrazine	$C_8H_{12}N_2$	13360-65-1	1.03 (27)	-	-	-	42.45 (60)	0.28 (27)	44.28 (22)	0.26 (17)
425	450	0.790	Tetramethylpyrazine	$C_8H_{12}N_2$	1124-11-4	-	-	-	-	2.35 (12)	-	2.03 (12)	0.03 (17)
426	455	0.800	2-Methyl-5-propylpyrazine	$C_8H_{12}N_2$	29461-03-8	-	-	-	-	-	-	0.65 (24)	-
427	470	0.860	2-Pentylpyrazine	$C_9H_{14}N_2$	-	-	-	-	-	0.32 (16)	-	-	-
428	500	0.740	2-Isobutyl-3-methylpyrazine	$C_9H_{14}N_2$	13925-06-9	-	-	-	-	0.82 (27)	-	-	-
429	515	0.690	3,5-Diethyl-2-methylpyrazine	$C_9H_{14}N_2$	18138-05-1	-	-	-	-	6.20 (24)	0.03 (32)	5.51 (14)	0.03 (24)
430	545	0.770	2-Butyl-3-methylpyrazine	$C_9H_{14}N_2$	15987-00-5	-	-	-	-	2.14 (25)	-	2.77 (19)	0.01 (43)
431	550	0.670	2,5-Dimethyl-3-isobutylpyrazine	$C_{10}H_{16}N_2$	32736-94-0	-	-	-	-	6.03 (33)	0.04 (37)	5.07 (3)	0.03 (31)
432	595	0.710	2-Butyl-3,5-dimethylpyrazine	$C_{10}H_{16}N_2$	50888-63-6	-	-	-	-	7.26 (37)	0.03 (40)	5.92 (11)	0.03 (20)
433	600	0.750	2-Methyl-3-propylpyrazine	$C_8H_{12}N_2$	15986-80-8	-	-	-	-	-	-	-	0.03 (39)
434	610	0.620	2,3-Diethyl-5-methylpyrazine	$C_9H_{14}N_2$	18138-04-0	-	-	0.49 (21)	-	-	-	-	-
435	615	0.630	2-(2-Methylpropyl)-3-(1-methylethyl)pyrazine	$C_{11}H_{18}N_2$	-	-	-	0.17 (21)	0.03 (19)	-	-	-	-
436	635	0.660	2,6-Dimethyl-3(2-methyl-1-butyl)pyrazine	$C_{11}H_{18}N_2$	56617-70-0	-	-	-	-	2.78 (44)	-	-	0.01 (33)

437	645	0.680	2,5-Dimethyl-3-(3-methylbutyl)pyrazine	$C_{11}H_{18}N_2$	18433-98-2	-	-	0.01 (33)	-	11.32 (40)	-	12.19 (12)	0.06 (25)
438	660	0.660	2,3,5-Trimethyl-6-butylpyrazine	$C_{11}H_{18}N_2$	10132-38-4	-	-	-		0.94 (31)	-	-	-
439	675	0.620	3,6-Dipropyl-2,5-dimethylpyrazine	$C_{12}H_{20}N_2$		-	-	0.23 (8)	0.05 (42)	-	-	-	-
440	680	0.630	1-(5-(2-Methyl-1-propyl)-2-pyrazinyl)-1-propanone	$C_{11}H_{16}N_2O$	86461-72-5	-	-	0.14 (14)	0.03 (32)	-	-	-	-
441	705	0.650	2-(3-Methylbutyl)-3,5,6-trimethylpyrazine	$C_{12}H_{20}N_2$	-	-	-	-	-	-	-	0.58 (35)	-
					Subtotal (GC Peak Area)	10.36	14.12	1.42	0.20	354.96	4.66	384.81	3.32
					Subtotal (%)	0.17	0.14	0.53	0.56	6.05	4.62	4.53	4.23
			S-compounds										
442	150	1.260	Thiazole	C_3H_3NS	288-47-1	-	1.54 (35)	-	-	10.76 (18)	0.19 (7)	9.01 (23)	0.22 (24)
443	155	0.620	Dimethyldisulfide	$C_2H_6S_2$	624-92-0	0.46 (42)	0.29 (36)	-	-	3.51 (8)	0.02 (8)	1.90 (36)	0.04 (11)
444	170	0.640	2-Methylthiophene	C_5H_6S	554-14-3	-	-	-	-	0.76 (18)	-	-	-
445	200	0.960	2-Methylthiazole	C_4H_5NS	3581-87-1	-	-	-	-	1.64 (20)	0.02 (12)	1.70 (15)	0.02 (25)
446	285	1.540	3-(Methylthio)-propanal	C_4H_8OS	3268-49-3	-	-	-	-	2.48 (31)	-	3.48 (7)	-
447	340	0.920	Dimethyltrisulfide	$C_2H_6S_3$	3658-80-8	-	0.26 (38)	-	-	1.79 (13)	-	1.01 (53)	0.04 (9)
448	390	1.850	2-Acetylthiazole	C_5H_5NOS	24295-03-2	-	-	-	-	21.32 (11)	0.13 (18)	17.47 (16)	0.07 (38)
449	425	0.650	1-(3-Thienyl)-2-propanone	C_7H_8OS	-	-	0.12 (32)	-	-	-	-	-	-
450	445	1.310	Benzenemethanethiol	C_7H_8S	100-53-8	-	-	-	-	0.85 (36)	0.02 (27)	-	-
451	480	1.530	2-Propionylthiazole	C_6H_7NOS	-	-	-	-	-	1.25 (36)	-	0.87 (28)	-
452	520	1.080	[(Methylthio)methyl]-benzene	$C_8H_{10}S$	766-92-7	-	-	-	-	-	-	0.42 (48)	-
453	545	1.440	Benzo[b]thiophene	C_8H_6S	95-15-8	-	0.45 (19)	-	0.0045 (38)	-	-	-	-
454	575	1.960	Benzothiazole	C_7H_5NS	95-16-9	4.04 (38)	28.81 (43)	0.18 (45)	0.13 (27)	0.81 (47)	0.15 (10)	8.74 (20)	-
455	580	0.880	Cyclohexyl isothiocyanate	$C_7H_{11}NS$	1122-82-3	-	0.96 (58)	-	0.01 (46)	-	0.01 (12)	-	-
456	590	0.660	2-Tertiobutylthiophene	$C_8H_{12}S$	-	-	-	-	-	1.52 (5)	-	4.22 (20)	0.03 (47)
457	675	0.420	Cyclohexylmethylbutyl sulfite	$C_{11}H_{22}O_3S$	-	-	-	0.10 (29)	0.01 (4)	-	-	-	-
458	680	0.540	S-Methyl(E)-oct-2-enethioate	$C_9H_{16}OS$	91944-66-0	-	-	-	-	-	-	0.28 (10)	-
459	950	0.460	Cyclohexylmethyl hexyl sulfite	$C_{13}H_{26}O_3S$	-	-	-	-	-	0.43 (28)	-	-	-
					Subtotal (GC Peak Area)	4.51	32.44	0.28	0.15	47.13	0.54	49.10	0.44
					Subtotal (%)	0.07	0.31	0.10	0.42	0.80	0.53	0.58	0.56
			Terpenic compounds										
			C10 Monoterpenenic compounds										
			Hydrocarbon-type										
460	310	0.410	α-Pinene	$C_{10}H_{16}$	7785-26-4	-	-	-	0.0050 (28)	-	0.01 (6)	-	0.01 (14)
461	330	0.480	Verbenene	$C_{10}H_{14}$	4080-46-0	-		-	-	-	0.01 (16)	-	-
462	375	0.480	α-Phellandrene	$C_{10}H_{16}$	99-83-2	-	0.92 (51)	-	0.0038 (10)	-	0.01 (48)	1.54 (10)	-
463	395	0.500	Limonene	$C_{10}H_{16}$	138-86-3	-	-	-	-	7.11 (16)	-	-	0.11 (47)
464	450	0.610	Bicyclo[3.2.1]oct-2-ene, 3-methyl-4-methylene-	$C_{10}H_{14}$	49826-53-1	-	-	-	-	-	-	-	0.07 (34)
465	485	0.790	2,6-Dimethylbicyclo[3.2.1]octane	$C_{10}H_{18}$	-	1.06 (33)	3.21 (25)	0.06 (82)	0.02 (6)	-	0.01 (16)	-	-
466	695	0.680	Bicyclo[3.1.1]heptane, 6,6-dimethyl-3-methylene-	$C_{10}H_{16}$	16022-04-1	-	2.37 (46)	-	-	-	-	-	-
			Oxygen-containg compounds										
467	400	0.500	1,8-Cineole	$C_{10}H_{18}O$	470-82-6	6.34 (10)	7.81 (46)	0.39 (10)	0.03 (36)	-	-	-	-
468	460	0.670	Dihydrolinalool	$C_{10}H_{22}O$	78-69-3	3.06 (55)	6.26 (32)	0.17 (21)	0.05 (19)	5.20 (21)	-	4.13 (22)	0.08 (49)
469	465	0.910	Linalool	$C_{10}H_{18}O$	78-70-6	2.36 (27)	7.06 (43)	0.28 (44)	0.07 (33)	5.76 (37)	0.21 (20)	-	-
470	480	0.940	Fenchyl alcohol	$C_{10}H_{18}O$	1632-73-1	0.43 (18)	-	-	0.06 (23)	4.46(13)	0.05 (49)	-	-
471	500	1.090	Pinocarveol	$C_{10}H_{16}O$	547-61-5	-	5.53 (14)	-	-	-	0.04 (22)	-	-
472	505	0.780	Camphor	$C_{10}H_{16}O$	464-48-2	3.23 (40)	6.42 (38)	0.17 (21)	0.02 (18)	-	0.03 (33)	1.46 (29)	-
473	505	1.150	Verbenol	$C_{10}H_{16}O$	473-67-6	-	-	-	0.07 (35)	-	-	-	-
474	510	0.670	p-Menthan-3-one	$C_{10}H_{18}O$	491-07-6	-	1.65 (29)	0.06 (42)	0.02 (27)	-	-	-	-
475	520	0.840	Pinocarvone	$C_{10}H_{14}O$	30460-92-5	-	-	-	0.03 (11)	-	-	-	-
476	525	1.120	Borneol	$C_{10}H_{18}O$	507-70-0	5.52 (41)	13.63 (25)	0.25 (45)	0.14 (27)	-	-	0.63 (56)	-
477	530	0.920	Menthol	$C_{10}H_{20}O$	2216-51-5	3.96 (41)	24.61 (9)	-	0.26 (22)	10.27 (27)	0.15 (38)	1.86 (10)	0.09 (31)
478	540	1.660	p-Cymen-8-ol	$C_{10}H_{14}O$	1197-01-9	-	6.73 (38)	-	0.07 (23)	-	-	-	-
479	545	0.910	Dihydrocitronellol	$C_{10}H_{22}O$	106-21-8	5.35 (30)	44.11 (28)	-	-	-	-	-	-

480	545	1.020	α-Terpineol	$C_{10}H_{18}O$	98-55-5	7.60 (9)	11.92 (47)	0.36 (50)	0.10 (20)	1.01 (23)	0.14 (13)	1.53 (36)	-
481	550	0.870	Myrtenal	$C_{10}H_{14}O$	564-94-3	-	4.23 (27)	-	0.03 (26)	-	0.06 (33)	-	-
482	560	1.040	Verbenone	$C_{10}H_{14}O$	1196-01-6	4.01 (16)	5.94 (37)	0.78 (21)	0.06 (30)	-	0.08 (25)	-	0.24 (35)
483	595	0.620	Linalyl acetate	$C_{12}H_{20}O_2$	115-95-7	-	3.15 (47)	-	0.01 (49)	-	0.06 (17)	-	-
484	625	0.630	Endobornyl acetate	$C_{12}H_{20}O_2$	76-49-3	9.34 (50)	12.37 (40)	0.48 (35)	0.09 (20)	3.49 (41)	0.08 (35)	8.53 (47)	0.09 (21)
485	675	0.680	β-Terpenyl acetate	$C_{12}H_{20}O_2$	10198-23-9	-	4.59 (48)	-	0.03 (19)	4.72 (15)	0.08 (14)	-	-
486	700	0.730	Geraniol acetate	$C_{12}H_{20}O_2$	105-87-3	-	6.05 (8)	-	-	-	-	-	-
487	735	0.910	Verdyl acetate	$C_{12}H_{16}O_2$	5413-60-5	-	3.93 (41)	-	0.02 (12)	4.11 (37)	0.04 (13)	3.87 (12)	-
					Subtotal (GC Peak Area)	52.27	182.50	3.00	1.19	46.13	1.04	23.56	0.69
					Subtotal (%)	0.85	1.76	1.12	3.31	0.79	1.03	0.28	0.88
			C15 Sesquiterpenes										
			Hydrocarbon-type										
488	725	0.530	Valencene	$C_{15}H_{24}$	4630-07-3	-	2.45 (22)	-	-	-	-	-	-
489	725	0.540	Longifolene	$C_{15}H_{24}$	475-20-7	-	-	-	0.02 (44)	-	0.05 (14)	-	-
490	820	0.600	δ-Cadinene	$C_{15}H_{24}$	483-76-1	-	0.66 (19)	-	-	-	-	-	-
491	820	0.690	Calamenene	$C_{15}H_{22}$	483-77-2	0.66 (39)	1.41 (43)	0.03 (28)	0.01 (34)	-	-	0.97 (16)	0.01 (14)
492	820	0.830	α-Bisabolene	$C_{15}H_{24}$	29837-07-8	-	23.55 (28)	-	-	-	-	-	-
493	835	0.770	α-Calacorene	$C_{15}H_{20}$	21391-99-1	0.77 (34)	0.81 (38)	-	-	-	-	0.60 (37)	0.01 (50)
494	840	0.870	Patchulane	$C_{15}H_{26}$	19078-35-4	-	4.43 (31)	-	0.02 (32)	-	-	-	-
495	1100	0.670	4,5,9,10-Dehydro-isolongifolene	$C_{15}H_{20}$	-	0.81 (29)	5.96 (48)	0.04 (33)	0.02 (21)	-	-	-	-
			Oxygen-containg compounds										
496	850	0.920	Nerolidol	$C_{15}H_{26}O$	7212-44-4	9.51 (28)	-	-	-	4.33 (30)	-	5.87 (47)	-
497	855	0.960	Longicamphenylone	$C_{14}H_{22}O$	-	-	14.93 (33)	0.24 (50)	0.04 (10)	-	-	-	-
498	880	0.780	Torreyol	$C_{15}H_{26}O$	19435-97-3	7.35 (39)		0.27 (44)	0.08 (15)	2.54 (20)	0.08 (18)	8.60 (24)	0.08 (15)
499	890	1.000	Cedrol	$C_{15}H_{26}O$	77-53-2	4.01 (27)	10.05 (40)	0.21 (18)	0.03 (33)	-	0.07 (44)	-	-
500	920	1.020	τ-Cadinol	$C_{15}H_{26}O$	5937-11-1	-	3.25 (39)	-	-	-	-	-	-
501	1175	0.820	α-Bisabolene epoxide	$C_{15}H_{24}O$	-	-	5.29 (47)	-	-	-	-	-	-
					Subtotal (GC Peak Area)	23.11	85.27	0.79	0.22	6.86	0.20	16.05	0.10
					Subtotal (%)	0.38	0.82	0.29	0.61	0.12	0.20	0.19	0.13
			C_{13} Norisoprenoid										
502	575	1.080	Tetrahydroionol	$C_{13}H_{26}O$	4361-23-3	-	-	-	0.02 (41)	-	-	-	-
503	645	0.610	Edulan	$C_{13}H_{20}O$	41678-30-2	-	-	-	-	-	0.06 (30)	-	-
504	780	0.750	α-iso-methyl ionone	$C_{14}H_{22}O$	127-51-5	2.71 (39)	4.36 (22)	0.14 (26)	0.02 (15)	2.31 (50)	0.04 (26)	3.65 (12)	0.03 (18)
					Subtotal (GC Peak Area)	2.71	4.36	0.14	0.04	2.31	0.10	3.65	0.03
					Subtotal (%)	0.04	0.04	0.05	0.11	0.04	0.10	0.04	0.04
					Total	6141.92	10373.49	268.68	35.91	5866.33	100.96	8502.26	78.51

[a] Retention times for first (^1t_R) and second (^2t_R) dimensions in seconds.
[b] Mean of three independent assays (n=3).